WILDLIFE

4.0 Introduction

Considerable progress has been made in identifying unique life history traits that may increase the risk of EDC exposure to wildlife compared with traditional laboratory animal models or humans. In fact, consideration that wildlife may be uniquely vulnerable to certain EDCs is justified for several reasons. It is conceivable that chemicals not identified as EDCs based on the responses of laboratory animal models may affect the endocrine systems of species that differ considerably. As well, certain aspects of the life history of a species may be uniquely sensitive (e.g., critical periods of development) to EDCs. Other aspects of their life history may predispose certain species through avenues (e.g., food source, habitat contamination levels) that increase their risk of chemical exposures that differ either qualitatively or quantitatively from other animals.

There are aspects of the endocrinology of vertebrates, such as key hormonally-regulated events involved in reproduction and development, that show little difference between wildlife species and animal models or humans (Norris, 1996; Ankley et al., 1998a; Van Der Kraak et al., 1998a). As of yet, and perhaps not surprisingly, research efforts have yielded inconsequential evidence to support the idea that vertebrate wildlife are inherently more sensitive to EDCs than laboratory animal models or humans. However, despite recent progress made in the understanding of the endocrine systems of some wildlife species, conclusions in this regard are hindered by current knowledge gaps of the biological processes of many wildlife species in question.

Vertebrate wildlife species are not the only animals that may be adversely affected by EDCs. The potential risks of EDCs to invertebrates warrant special consideration given the highly divergent nature of endocrine systems within these taxa, which also differ markedly compared with vertebrates (LeBlanc et al., 1999). For example, invertebrate endocrine systems operate with different proteins, as well as hormones unique to these taxa, like terpenoids and ecdysteroids (e.g. ecdysone, 20-hydroxyecdysone). Homosesquiterpenoid epoxides and methyl farnesoate, which serve as juvenile hormones in insects and crustaceans, respectively, are other examples of hormones not found in vertebrates (Cymborowski, 1992; Laufer et al., 1993). These obvious differences make it apparent that responses to environmental chemicals in invertebrates cannot be predicted based on the endocrine responses of traditional laboratory animal models or humans.

One aspect of life history that is common to a wide array of wildlife species and not displayed in common animal models is oviparity. Oviparous species require unique consideration in terms of exposure during early development. An extensive review of the consequences of oviparity in the context of exposure characterization for EDCs was developed by Kleinow et al. (1999); only a few of the more salient points of their analysis are covered here. First, as opposed to viviparous species, the chemical dose to which early developmental stages of oviparous animals are exposed is derived largely via maternal transfer associated with deposition of nutrients to the maturing oocytes. Therefore, although the egg affords some degree of protection from contaminants in the external environment, it also can subject embryos to elevated, maternally derived, chemical exposures during potentially sensitive early developmental stages. A first-order approximation of the initial exposure received by oviparous embryos can be obtained by assuming a lipid-normalized relationship between contaminants in the maternal organism versus those in her eggs (Russell et al., 1999). However, this relationship has only been characterized for relatively stable, nonionic organic chemicals. Furthermore, the closed environment of the egg (i.e., little excretion) and the minimal biotransformation abilities of young embryos create unpredictable exposure variations (in terms of toxicokinetics) as the animals develop. Contaminants also partition from compartments associated with nutrient storage to developing tissues. The degree to which oviparity is an important and/or unique variable with respect to predicting EDC effects will depend upon the physico-chemical characteristics of the contaminant of concern, and the mechanism(s) of action (MOA) under consideration

There are other aspects of life history that differ in wildlife species that may complicate efforts to generalize the potential impacts of EDCs across species. For example, there are certain developmental events that are unique to specific species or classes of vertebrates such as external metamorphosis in amphibians, marine flatfish, and agnathans (e.g., lamprey), or smoltification in salmonids. In addition, processes unique to some taxa of invertebrates (i.e., molting, limb regeneration, diapause, pheromone production, pigmentation, metamorphosis) are also under endocrine control. In general, wildlife studies have not considered the implications of timing of exposure to EDCs relative to these critical aspects of reproduction or development.

This chapter evaluates the extent to which wildlife species, including both vertebrates and invertebrates, have been affected by environmental compounds whose primary mode of action involves endocrine disruption. This includes a consideration of the unique aspects that make the various taxa susceptible to the potential effects of EDCs. Subsequently, field observations and supporting laboratory data are critically evaluated with respect to the plausibility that responses are linked to exposure to

substances that function as EDCs. The intention here is not to provide an extensive review of all examples of alterations in reproduction and development that have been attributed to chemical exposures. Rather, the approach is to focus on selected cases studies that are sufficiently developed that they may ultimately be evaluated using a weight of evidence approach (Hill, 1965; Fox, 1991; Ankley and Giesy, 1998). Finally, some of the uncertainties and data gaps that confound our understanding of the extent to which wildlife species have been and may continue to be impacted by exposure to EDCs are considered.

4.1 Mammals

4.1.1 Unique aspects

Current day mammals are represented by over 4,500 species and so it is not surprising that they possess a wide array of life history strategies (Vaughan et al., 2000). In monotremes, represented only by the duck-billed platypus and the echidna, females lay leathery-shelled eggs like those of turtles. In marsupials, such as kangaroos, wombats and opossums, embryos develop in the uterus until they have enough musculature and coordination to move by themselves. The young then leave the uterus, crawl into the mother's pouch and continue their development attached to one of her mammary glands. However, the vast majority of mammalian species have young that develop inside the uterus attached to a placenta. As all species of mammals suckle their young, this represents an important avenue for exposure to chemical contaminants during critical periods of development.

Fish-eating mammals may be particularly vulnerable to EDCs because of (1) their position in the food chain, (2) dependence on an aquatic/marine foodweb rather than a terrestrial foodweb, (3) occurrence in areas influenced by industry and agriculture, and (4) specific aspects of their reproductive physiology. As high trophic consumers, these species often acquire large burdens of persistent pollutants through the biomagnification of compounds obtained from contaminated prey, including many potential EDCs (Tanabe et al., 1988). Pinnipeds and cetaceans have relatively large amounts of blubber for insulation and readily bioaccumulate highly persistent OC insecticides, PCBs and other lipid-soluble compounds. Indeed, both reproductive and non-reproductive toxicity has been found in many fish-eating mammalian species that live in riverine or coastal areas where contaminant burdens are generally higher than in more open ocean or pelagic systems. Some of the best evidence for a relationship between OCs and reproductive toxicity stems from field studies on seals (Bergman and Olsson, 1985; Reijnders, 1986) and mustelids (Wren, 1991, Kihlström et al., 1992, Leonards, 1997). These species exhibit the phenomenon of embryonic diapause, which may be a critical phase in the reproductive cycle that is prone to disruption by contaminants (Reijnders and Brasseur, 1992).

Feeding strategy may directly impact exposure to EDCs. For example, studies by Talmage and Walton (1991), which compared the contaminant burdens of rodents, demonstrated that insectivores (shrews) had the highest contaminant burden, followed by omnivores (cricetid mice) and finally, herbivores (voles). But herbivores may be uniquely exposed to plant derived compounds that impact the endocrine system. For example, the steroid alkaloid plant toxins (jervine, 11-deoxjervine and 3-O-glucosyl 11-deoxyjervine) were demonstrated to cause teratogenesis in sheep grazing on the range plant, *Veratrum californicum* in Western North America (Omnell et al., 1990; Cooper et al., 1998). There are numerous other examples of plant-derived compounds that alter various aspects of development (Keeler and Panter, 1989; Bunch et al., 1992). However, establishing whether or not these compounds affect endocrine function, and if so, are these effects manifested in wildlife, requires further investigation.

4.1.2 Effect based responses and case studies

4.1.2.1 Reproductive dysfunction in mustelids

Populations of mustelids, including mink (*Mustela vison*) and Canadian otter (*Lutra canadensis*), have declined in regions of the Great Lakes where these species have a high content of fish in their diet (Wren, 1986; Wren, 1991). Because Great Lake's fish contain elevated concentrations of numerous synthetic OCs including pesticides and PCBs, it is plausible to hypothesize a contaminant-based etiology. Several studies have shown that ranched mink that are fed a diet of Great Lakes' fishes, predominantly salmonids, result in adverse reproductive outcomes (Ankley and Giesy, 1998). Of all the pollutants that Great Lakes' mink may be exposed to, PCBs and to a lesser extent, TCDD, have the greatest impact (Giesy et al., 1994a). Yet, data are not available that would provide conclusive evidence that these compounds are linked to effects at the population level of wild mink. In particular, data that link wild mink exposed to persistent OCs, such as PCBs, PCDFs, and PCDDs, and changes in population dynamics or endocrine dysfunctions are missing. In the absence of an understanding of the underlying mechanisms mediating these effects, the link between these compounds and endocrine function is tentative at best.

A recent study using mink demonstrated that exposure to low doses of PCBs over 18 months, including two reproductive seasons, impaired reproduction (Brunstrom et al., 2001). The observed effects included fetal deaths, abnormalities, decreased kit survival, and decreased kit growth. The authors state that the doses of Clophen A50 (a technical PCB preparation) given are relevant in terms of wildlife PCB-exposure via the diet in contaminated areas. Furthermore, the reproductive dysfunction was found to involve PCB congeners that are Ah receptor agonists (Brunstrom et al., 2001).

Further evidence that mustelids may be sensitive to environmental compounds comes from a study of river otter on the Columbia River in the northwest United States. In this case, there were reports of reduced baculum length and weight and no evidence of spermatogenesis in 8 to 10 month old individuals (Henny et al., 1996). These effects seem to result from delayed development and are transient, as they do not persist into adulthood. These responses were correlated with a number of OC insecticides, PCBs, dioxins, and furans. In addition, European otter (*Lutra lutra*) populations in Europe declined dramatically during the 1960s to 1980s, and exposure to PCBs that result in impaired reproduction has been considered to be a major cause of this decline (Kihlström et al., 1992; Leonards, 1997; Brunstrom et al., 1998; Roos et al., 2001). A recent study by Roos et al. (2001) on Swedish otter populations concluded that these populations have increased during the 1990s. This improvement coincides with a decrease of PCB concentrations in the Swedish environment; supporting the idea that PCB contamination has been the lead cause of the declines of European otter populations in the past decades (Roos et al., 2001). However at this time, there is inadequate population data providing a link between exposure and reproductive outcome and insufficient knowledge on the impact of other environmental stresses.

4.1.2.2 Reproductive dysfunction in marine mammals

Harbour seals (*Phoca vitulina*) in the Dutch Wadden Sea exhibited low reproductive success and declining population numbers that were attributed to the impact of PCBs (Reijnders, 1980). Comparative population studies showed that the observed population decline (66%) could be fully accounted for by the observed decreased reproduction. Other studies showed that female harbour seals fed fish from the polluted Wadden Sea exhibited a lower reproductive success (50%) than the seals fed less contaminated fish from the Atlantic. In the same study, implantation failure was found to be associated with lower levels of 17β-estradiol (E_2) (Reijnders, 1986; Reijnders, 1990). Despite the strong correlation with OC exposure, there is still an incomplete understanding of the specific compounds responsible for the pathological effects and their mechanism of action(s) (Troisi and Mason, 1998; Reijnders, 1999). It is plausible that PCB-induced reductions in E_2 levels are the result of alterations in enzyme metabolism (Reijnders, 1999). This hypothesis is based on the observation that induction of the P450-isoenzyme CYP1A(2) causes increased hydroxylation of E_2 (Funae and Imaoka, 1993), combined with the findings that CYP1A(2) is significantly induced in PCB-exposed harbour seals (Boon et al., 1987). More detailed studies are required to substantiate this hypothesis.

DeLong et al. (1973) found stillbirths and premature pupping in California sea lions (*Zalophus californiansus*) to be associated with high PCB and DDE levels. Further research revealed that diseases (e.g., leptospirosis and calcivirus infections) were prevalent in the sea lions that could also contribute to abortions, endocrine disorders and premature pupping (Gilmartin et al., 1976). As both the EDCs and the diseases could have the same effect, it is not possible to discern a cause and effect relationship between contaminant levels and effects on reproduction. More studies are needed to clarify the underlying mechanisms.

There is ample evidence that populations of Baltic ringed seals (*Phoca hispida botnica*) and grey seals (*Halichoerus grypus*) have declined markedly over the past 100 years (Helle, 1983; Bergman and Olsson 1985; ICES, 1992). While overhunting and habitat destruction may have been contributing factors, it is generally accepted that persistent pollutants, which adversely affected the reproductive performance of the females, resulted in the decline in seal numbers. Some of the disorders observed in exposed seals include interruptions during early pregnancy, stenosis and occlusions, and partial or complete sterility of Baltic ringed seals (70%) and grey seals (30%). A time trend study by Roos et al. (1998) examined PCB and DDT concentrations in 177 juvenile grey seals collected between 1969 and 1997 along the Swedish Baltic coast. Their results also implicate PCBs as the leading cause of the reproductive dysfunctions observed in Baltic grey seals. While it is evident that the Baltic seals exhibit a compromised endocrine system, which has been associated with high PCB and DDE/DDT levels, further research is needed to elucidate the mechanism of action leading to impaired reproductive performance.

4.1.2.3 Reproductive dysfunction in feral rodents

Adult male white footed mice (*Peromyscus leucopus*) inhabiting a PCB- and cadmium-contaminated area had significantly lower relative testis weights compared with mice collected at a non-polluted site (Batty et al., 1990). Seminal vesicle weights in animals from both areas were not significantly different, but there was a higher variability in animals from the contaminated site. The responses of contaminant-exposed individuals were reflected at the population level as the proportion of juveniles and sub-adults in the summer population did not increase in the PCB- and cadmium-polluted area compared with an increase in the unexposed reference population (Batty et al., 1990). Further research is needed to determine whether these effects can be attributed to PCBs and/or cadmium and if the mechanisms contributing to the observed reproductive effects involve endocrine disruption.

A number of other studies have indicated a contaminant-associated effect on reproduction in wild populations of small rodents. White footed mice inhabiting a low PCB-contaminated woodland exhibited a higher population density but greater temporal variability between years and a higher number of transient mice compared with mice from an uncontaminated area (Linzey and Grant, 1994). Meadow voles (*Microtus pennsylvanicus*) inhabiting a chemical waste site at Niagara Falls, showed significantly reduced population density and mean life expectancy, as well as liver, adrenal and seminal vesicle weight compared with animals from a reference site. Tissues of voles from the waste site contained HCH and other chlorinated hydrocarbons. These were not found in tissues of voles from the reference site (Rowley et al., 1983). In a large-scale semi-field study, Pomeroy and Barrett (1975) found that application of a carbamate insecticide, Sevin, contributed to delayed reproduction and reduced recruitment in cotton rats (*Sigmodon hispidus*). While these studies indicate that small rodent populations may experience adverse reproductive effects as a result of exposure to environmental chemicals, more research is required to define the mechanism(s) of toxicity.

4.1.2.4 Less developed case studies

In addition to the species discussed so far, there are numerous others that seem to show signs of endocrine disruption or other adverse physiological effects as a result of exposure to substances with endocrine disrupting properties. In these cases, however, only inconclusive or circumstantial data is available, which obscure potential links between EDC exposure and fitness in these animals. The following brief examples highlight the wide range of potential effects, which encompass both reproductive and non-reproductive dysfunction (i.e., pathological disorders and altered immunity).

4.1.2.4.1 Reproductive effects

A notable example includes the remaining endangered Florida panthers (*Felix concolor coryi*) that exhibit a variety of defects of the reproductive, endocrine and immune systems (Facemire et al., 1995). These include a high prevalence of sperm abnormalities, low sperm density, thyroid dysfunction, sterility, and cryptochordism (90% of the male population).

In Alberta, Canada, there are incidences of masculinization (i.e., pseudohermaphroditism) of black and brown bears, but the cause remains unknown (Cattet, 1988). Pseudohermaphroditism was observed in both young (6 months) and old (14 years) female bears. Given the frequency of occurrence, it has been suggested that this effect may be exogenously induced (Cattet, 1988). The herbivorous habits of

black and brown bears may expose them to teratogenic herbicides or plant-derived alkaloids that are androgenic. It is also possible that the pseudohermaphroditism is the result of endogenous factors (i.e., excessive maternal androgens) (Benirschke, 1981). Similarly, female polar bears (4 of 269) from Svalbard, Spitsbergen were described as pseudohermaphrodites by Wiig et al. (1998). This distinction was based on the presence of a 20 mm penis containing a baculum in two of the bears, while the other two bears exhibited aberrant genital morphology and a high degree of clitoral hypertrophy. It is also known that PCB levels are high in polar bears from Svalbard (Bernhoft et al., 1997), but no link has been made between the contaminant and endocrine function.

There are numerous other examples suggesting that exposure to OC insecticides and PCBs have affected endocrine function and reproduction in marine mammals. For example, transformation of epididymal and testicular tissue has been observed in North Pacific minke whales (*Balaenoptera acutorostrata*) (Fujise et al., 1998). Hermaphroditism, in a limited numbers of cases, has been observed in beluga whales (*Delphinapterus leucas*) in the St. Lawrence River (De Guise et al., 1994). This has been attributed to PCB/DDT-related hormonal disturbances during stages of early pregnancy, whereby normal differentiation of male and female organs was disrupted. Subramanian et al. (1987) reported an inverse relationship between plasma testosterone levels and DDE (but not PCB) concentrations in the blubber of Dall's porpoises from the Northwestern Pacific Ocean.

4.1.2.4.2 Non-reproductive effects

Pathological lesions have been reported in several wildlife species that have been exposed to contaminants with known endocrine disruptive properties. For example, severe adrenocortical hyperplasia, osteoporosis, intestinal ulcers, claw malformations, arteriosclerosis, uterine cell tumours and decreased epidermal thickness have been reported in Baltic ringed and grey seals (Bergman and Olsson, 1985; Bergman 1999a, b). These effects have been attributed to PCBs, DDT and their metabolites, notably PCB-, DDE-methyl sulfones and DDD (Lund, 1994), which affect the function of the hypothalamic-pituitary-gonadal and adrenal axis. Pathological disorders, such as adrenal hyperplasia and high incidence of neoplasia, have been reported in beluga whales (*Delphinapterus leucas*) in the St. Lawrence River (De Guise et al., 1994, Martineau et al., 1994). Although these effects were associated with exposure to PHAHs and PCBs, the involvement of these contaminants in the etiology of the disorders is uncertain.

There is considerable circumstantial and experimental evidence concerning the impact of contaminants with endocrine disrupting properties on immune function in mammalian wildlife. A confounding factor in discerning whether or not contaminant-induced immune dysfunction results from endocrine disruption is the intricately connected nature of these two systems. Consequently, there is little concrete evidence to support this hypothesis (Reijnders, 1999). One example where this mechanism might be involved is the contaminant-induced immune suppression that has been proposed to contribute to the mass mortalities of marine mammals including the harbour seal, Baikal seal (*Phoca sibirica*), striped dolphin (*Stenella coeruleoalba*), and bottlenose dolphin (*Tursiops truncata*) (Dietz et al., 1989). In a controlled experiment, female harbour seals fed fish from the polluted Wadden Sea had impaired natural killer cell activity and T-lymphocyte function (de Swart et al., 1994), as well as delayed-type hypersensitivity (Ross et al., 1995) compared with seals fed less contaminated fish from the Atlantic. Although this study showed that contaminants in the fish were immunotoxic, the significance of this finding in terms of impact on survival in the wild is unknown

4.1.3 Conclusion

The current state of the science provides sufficient evidence that feral mammals have been adversely impacted by environmental contaminants but limited evidence exists to support the contention that these effects are mediated through endocrine-dependent mechanisms. Several factors make it difficult to assess the mode(s) of action of environmental chemicals in feral mammals, including a general lack of knowledge regarding their endocrinology/reproductive biology, and how other environmental stressors affect these processes.

4.2 Birds

4.2.1 Unique aspects

There are several aspects of the biology of birds that may make them uniquely vulnerable to potential EDCs. Birds have high metabolic rates and on a weight-adjusted basis often have a higher metabolism and food consumption than placental mammals of similar size. These factors, together with increased rates of metabolic biotransformation of xenobiotics, may contribute to an increased exposure to environmental contaminants. Migration, courtship, breeding and parental care giving behaviours require high expenditures of energy and are often accompanied by periods of starvation. Birds respond to these

situations by mobilizing stored lipid thereby raising the potential of increased exposure to lipophilic contaminants that are subsequently released.

Bird species vary in terms of the extent of development of the young at hatching. Birds that are in an advanced state of development are called precocial, whereas those in an early stage of development are termed altricial. Relative to the size of the adult bird, the eggs of altricial species are smaller than those of precocial species and contain less yolk. Altricial species thermoregulate upon hatching and therefore utilize a higher percentage of ingested energy for growth than precocial birds. In contrast, the demands on the adults of altricial species may be greater due to the relatively narrower limits for brooding behaviour (Kleinow et al., 1999). These two developmental strategies could have profound toxicological and toxicokinetic consequences.

There are aspects of sexual differentiation in birds that may make them uniquely sensitive to the effects of EDCs with estrogenic activity. Exposure of an avian embryo to exogenous estrogens during a critical period in development may result in a more adverse effect than would exposure of a mammalian fetus to estrogens during the same critical period. This is explained by the differential roles of estrogens in birds compared with mammals. In birds, estrogen is the differentiating hormone for both the gonads and behaviour, but is not involved in the differentiation of the gonads in mammals. Estradiol, in conjunction with other endocrine and paracrine factors, is implicated in the unilateral development of the left ovary and regression of the right ovary. Also, estradiol influences whether the embryonic tissues that differentiate into oviducts and shell gland persist or regress (NRC, 1999). The dramatic sex-reversing effects of early estrogen treatment on male Japanese quail (*Coturnix coturnix japonica*) behavior only occur if the treatment is given before day 12 of the 18-day incubation period (Adkins, 1979), while the estradiol-induced masculinization of female zebra finch (*Taeniopygia guttata*) is only produced by treatment after hatching (Adkins-Regan et al., 1994). Japanese quail are a precocial species, while zebra finches are typical altricial songbirds. The timing of sexual differentiation of behavior in these two species is consistent with the observation that precocial and altricial birds develop similarly, but hatch at different stages of the overall developmental sequence (Adkins-Regan, 1987).

Carnivorous and especially piscivorous birds as a result of their feeding behaviour are exposed to a number of persistent and bioaccumulative organic compounds, many of which are halogenated (Giesy et al. 1994b). Specifically, it has been the persistent chlorinated hydrocarbons that have accumulated to the greatest concentrations and have been related to the most severe adverse effects on the reproductive

potential of birds (Kubiak et al. 1989), such as deformities and mortality of embryos (Gilbertson, 1983; Giesy et al., 1994b). These effects have contributed to population declines (Peakall, 1986; Peakall, 1988).

4.2.2 Effect based responses and case studies

4.2.2.1 Alterations in behaviour

There is evidence from both field and laboratory studies that environmental contaminants from the Great Lakes region influence behaviour and reproductive success of colonial waterbirds. Behavioural abnormalities observed in herring gulls (*Larus argentatus*) from Lake Ontario include aberrant parental behaviour involving a failure to sit on eggs or defend nests (Fox et al., 1978). It was suggested that the high levels of chemical contaminants in these birds were the causative factor for the abnormal behaviour.

OCs have altered avian reproductive behavior in controlled studies. Ring doves (*Streptopelia risoria*) that were fed a mixture of DDE, PCBs, mirex, and photomirex (contaminants that are found in salmon and gulls of Lake Ontario) exhibited alterations in hormone levels and reproductive behaviors as adults (McArthur et al., 1983). Consumption of the contaminated diets led to a reduction and/or delay in behaviorally-induced increases of sex hormones, contaminated females failed to respond to male courtship behaviors in the normal fashion, and pairs receiving the highest dosage spent less time feeding their young. There was a marked dose-related decrease in fledging success, and the breeding cycle was asynchronous in OC-treated birds. The administration of a PCB mixture to adult breeder doves resulted in aberrant incubation (Peakall and Peakall, 1973) and courtship (Tori and Peterle, 1983) behaviours. The PCB-dosed females were particularly affected in the later experiment and performed only a small number of courtship behaviors, which resulted in a severe impairment of reproductive success. Exposure to the organophosphorus insecticide parathion may also impact avian incubation behavior and reproductive success (White et al., 1983; Bennett et al., 1991).

Studies with Japanese quail and zebra finches provide substantial evidence that sexual behavioural differentiation in birds is sensitive to androgens and estrogens and that hormonal disturbances can have a profound and permanent change in the reproductive behaviour of both sexes (Adkins, 1979; Adkins-Regan, 1987; Simpson and Vicario, 1991; NRC, 1999). While this forms the underlying basis whereby environmental contaminants may modulate behaviour, the causative agents and underlying mechanisms responsible for changes in reproductive behaviour in wild bird populations is unknown.

4.2.2.2 Abnormal reproductive morphology

Gonadal development may be impacted in wild bird populations as a result of exposure to high levels of OCs. Fifty-seven percent of male gull *(Larus argentatus*) embryos collected from Scotch Bonnet Island, Canada in 1975 and 1976 had testicular feminization (Fox, 1992). Eggs from this site were contaminated with dioxins, PCBs and Mirex (Gilman et al., 1979; Fox, 1992). Similarly, a tern (*Sterna forsteri*) colony showed a high incidence of abnormal testes (Nisbet et al., 1996), but the contaminants responsible for these effects have not been identified. There is also uncertainty as to the interpretation of changes in gonadal morphology in birds as this may represent a normal condition that disappears with age. Additionally, there is little evidence that this widespread feminization is associated with population level effects.

Ovotestis formation in male embryos and retention of the right oviduct in female embryos was observed in experimental studies of gull eggs injected with estradiol, DES and environmental contaminants such as methoxychlor and DDT (NRC, 1999a). Altered gonadal development was observed following the injection of *o,p'*-DDT or methoxychlor into Western (*Larus occidentalis*) and California (*Larus californicus*) gull eggs on day 1 of incubation (Fry and Toone, 1981; Fry et al., 1987). The injection of methoxychlor and *o,p*'-DDT resulted in the feminization of gonads of male embryos and the persistence of right oviducts in surviving female embryos. These effects were intermediate between embryos from control eggs and eggs injected with estradiol. It is difficult to judge the functional significance of these results. The criteria for feminization were a small histological change (i.e., a localization of primordial germ cells in a thickened cortex on the surface of the left testis), and it is not clear whether this change would impact the reproductive success of adult birds. While these studies point to the possible involvement of the endocrine system, conclusive evidence is lacking.

An attempt to correlate gonadal feminization with OC contamination in a Glaucous gull (*Larus hyperboreus*) population in Puget Sound was inconclusive (Fry et al., 1987). A total of 31 adult females from several colonies that span a range of contamination levels were trapped on their nests and sacrificed for gonadal inspection. Interestingly, the lengths of right oviducts were correlated with predicted degrees of chemical contamination. However, the significance of these data is unclear, as all birds were successfully incubating clutches. Furthermore, the rating of the most severe category (greater than 10 mm) is actually similar to the size of a normally occurring vestigial right oviduct (9 to 10 mm) in the herring gull (Boss and Witschi, 1947), calling into question the relevance of this endpoint.

4.2.2.3 Sex ratio skew and female-female pairings in gull populations

There is evidence that the sex ratio has been affected in several North American gull populations resulting in an overabundance of females in some breeding colonies. Associated with changes in sex ratio, there has been an increase in the incidence of female-female pairings in regions contaminated by DDT (Fry et al., 1987; Fox, 1992). A dramatic example occurred in the Western gull population on Santa Barbara Island, California, from 1972 - 1978 (Hunt et al., 1980). The incidence of female-female pairings in a colony is usually estimated by documenting the number of nests that contain an abnormally large number of eggs, or 'supernormal clutches'. Some supernormal clutches arise from female-female pairs, while others are associated with polygynous trios of two females and one male (Conover, 1984a). Nests with five or greater eggs are usually the result of multiple-female associations (Conover et al., 1979), as a single female gull typically lays 1 to 3 eggs. An increase in supernormal clutch incidence was observed in herring gulls inhabiting Northeastern Lake Michigan during the period of 1978 to 1981 (Shugart, 1980; Fitch and Shugart, 1983). Both the California and Great Lakes populations of gulls were exposed to relatively great concentrations of OCs, including DDT, from the 1950s until the 1970s (Fry and Toone, 1981; Fry et al., 1987, Fox, 1992).

Several historical studies have investigated the occurrence of supernormal clutches in the Laridae family utilizing literature sources and museum specimens, in order to determine whether incidences have actually changed in the pre- and post-DDT era. It was discovered that the incidence of supernormal clutches has actually decreased significantly for many species of terns throughout the United States (Conover, 1984b). Supernormal clutch incidence had increased significantly in only three Laridae species since 1950: Western gulls and herring gulls nesting in the Great Lakes, and Caspian terns (*Hydroprogne caspia*) breeding in the United States. Supernormal clutches were a regular occurrence in Ring-billed (*Larus delawarensis*) and California gulls prior to the DDT era, and their occurrence has not changed over time (Conover and Hunt, 1984a). In contrast, supernormal clutches were not found regularly in Western or herring gulls until after 1950, and the sex ratio for their populations as a whole has changed dramatically for both species towards an excess of females. These results supported the hypothesis that the shortage of males at the breeding colonies resulted from a low male/female ratio in the adult population and not from a failure of feminized males to breed.

A decrease in the ratio of males to females in Western and herring gulls could be due to a differential mortality between males and females for each of the two species. Such a differential mortality has not been well studied. It is possible that male gulls could be more susceptible to poisoning

from persistent OC contaminants. Male Western gulls weigh about 25% more than females, on average, and they feed higher up on the food chain (Pierotti, 1981). Also, male gulls do not have the ability to excrete lipophilic contaminants by laying eggs. For these reasons, it is expected that male gulls might accumulate greater body burdens of these toxicants compared with females. Another possible explanation for the skewed sex ratios observed in gulls from California and the Great Lakes is that exposure to environmental estrogenic contaminants caused either differential male mortality or a feminization of male embryos, which resulted in chemical sterilization and a failed recruitment into the breeding population (Fry et al., 1987). While this is a plausible hypothesis, there is no direct evidence to support it or the mechanism(s) that involves endocrine disruption.

4.2.2.4 DDE-induced eggshell thinning

Toxicant-induced eggshell thinning, primarily caused by DDE (degradation product of DDT), can result in cracked or broken eggs and other adverse reproductive effects (Weseloh et al., 1983; Struger and Weseloh, 1985; Struger et al., 1985; Elliott et al., 1988). Eggshell thinning is mediated by direct effects of DDE on the shell gland (see Chapter 3 for discussion of possible mechanisms) and its occurrence during the period of use of DDT as an insecticide in North America nearly resulted in the extinction of several avian species. Studies in Canadian and Russian peregrine falcon (*Falco peregrinus*) suggest that eggshell thinning continues to be a problem due to the high DDT content in the eggs (Johnstone 1996; Henny et al. 1994; Johnstone et al. 1996).

The degree of eggshell thinning varies depending on the sensitivity of the species. For example, eggshell thickness is reduced by more than 30% in response to DDE in brown pelican (*Pelecanus occidentalis*), whereas Japanese quail eggshell thinning ranges from 5-15%. In certain species (e.g., domestic fowl, *Gallus domesticus*), DDE does not induce eggshell thinning. Many of the species susceptible to eggshell thinning, such as the guillemot (*Uria aalge*), double-crested cormorant (*Phalacrocorax auritus*), have experienced dramatic population increases and increased eggshell thickness (see Figure 4.1) since DDT was banned from use (Ludwig, 1984; Price and Weseloh, 1986; Bignert et al., 1994; Weseloh and Ewins, 1994). However, other adverse effects, such as localized impairment of reproductive performance (Tillitt et al., 1992) and anatomical defects (Giesy et al., 1994b, Yamashita et al., 1992) have persisted.

[insert Figure 4.1]

4.2.2.5 Deformities

A group of embryonic abnormalities directly related to contaminant exposure in some fish-eating birds has been defined as a specific syndrome, GLEMEDS (Great Lakes Embryo Mortality, Edema and Deformity Syndrome) (Gilbertson and Fox, 1977; Gilbertson et al., 1991). GLEMEDS involves a consistent pattern of subcutaneous edema, beak malformations, cardiac edema, and skeletal malformations (Fox et al., 1991; Ludwig et al., 1993; Gilbertson et al., 1991) and particularly, abnormalities that are of ectodermal origin (Rogan et al., 1988). An abnormality that has been characterized well in cormorants (crossed-bill syndrome) has been correlated with concentrations of different polychlorinated halogens (PCHs) in bird eggs (Fox et al., 1991). The expression of this syndrome is the result of the deposition of coplanar PCB congeners in the eggs by the maternal bioaccumulation of the compounds that were present in their fish-based diet (Gilbertson et al., 1991).

Declines in concentrations of DDT, PCBs, and PCDDs/Fs in the Great Lakes have been associated with increasing populations of herring gulls and double-crested cormorants, as well as other fish-eating birds. There have also been reductions in the rate of reproductive failure and the symptoms of GLEMEDS (Gilbertson et al., 1991; Grasman et al., 1998). Nevertheless, biochemical effects associated with persistent OC exposure persists in all species of fish-eating colonial water birds inhabiting Lake Ontario and the other Great Lakes (Fox et al., 1991; Fox, 1993). PCHs also effects the concentrations of both blood and liver levels of vitamin A. As vitamin A is necessary for normal embryonic development (Zile et al., 1997), it is possible that altered levels of vitamin A contribute to the birth defects in birds.

4.2.3 Conclusion

In birds, the oviparous reproductive strategy and certain life history traits create avenues of exposure that may make these species more vulnerable to EDCs than traditional animal models or humans. Although exposure to environmental contaminants can have dramatic effects (i.e., eggshell thinning) on endocrine-regulated processes (e.g., reproduction) and overall population fitness, the mechanism need not involve endocrine disruption. Likewise, the same individuals may experience endocrine disruption, which may or may not be linked to effects on reproduction and population fitness.

4.3 Reptiles

4.3.1 Unique aspects

Reptiles encompass a broad diversity of groups, including squamates (lizards and snakes), turtles and tortoises, crocodilians (crocodiles and alligators), and sphenodon (tuatara). Their evolutionary history has given them a suite of unique phylogenetic, anatomical, physiological and ecological characteristics that must be considered when evaluating the potential effects of environmental EDCs (Lamb et al., 1995; Palmer et al., 1997; Crain and Guillette, 1998). As well, endocrine systems are highly divergent across this class, which confounds efforts to make predictions of the physiological responses across reptilian species.

This phylogenetic background has led to a diversity of reproductive and developmental characteristics. For instance, although all tuatara, turtles and crocodilians are oviparous, the squamates exhibit both oviparity and viviparity (Palmer et al., 1997). Even among the oviparous species, there is significant variation in the anatomy of the female reproductive tracts (Palmer and Guillette, 1988; Palmer and Guillette, 1990; Palmer and Guillette, 1992). In all species of reptiles, offspring are formed as miniature copies of the adults, without larval stages. The eggs of most oviparous reptiles are buried, providing a route for embryonic exposure via the substrate. Although an eggshell surrounds the embryo, dissolved compounds readily cross into the egg.

Reptiles exhibit a variety of sex determining mechanisms, including genetic and environmental sex determination, such as temperature-dependent sex determination (TSD). TSD occurs among all crocodiles, most turtles, and many lizards (Lance, 1994). In TSD reptiles, the incubation temperature of the eggs determines whether the offspring will be male or female. However, there is tremendous variation in the pattern of TSD among reptiles (Wibbels et al., 1998). This variation complicates the understanding of potential influences that EDCs may have on the process of sex determination in reptiles.

Steroid hormones (e.g., estrogens, testosterone) have been shown to override the effects of temperature on sex determination in TSD species (Wibbels and Crews, 1995). For example, the administration of exogenous estrogens results in female sex determination, even though the eggs are incubated at all-male producing temperatures. Likewise, several environmental chemicals (e.g., PCBs, trans-Nonachlor, cis-Nonachlor, chlordane, and p,p'DDE) have been shown to alter turtle sex determination (Bergeron et al., 1994; Crews et al., 1995; Willingham and Crews, 1999). In addition, PCBs and chlordane also alter steroid hormone profiles of hatchling turtles (Willingham et al., 2000a).

The mechanism by which hormones and putative EDCs influence sexual determination remains unclear. Since testosterone serves as the precursor to both estradiol and DHT, it has been hypothesized that the TSD phenomenon in reptiles results from competition between 5α-reductase and aromatase (Crews and Bergeron, 1994; Jeyasuria and Place, 1998). Aromatase activity is associated with female producing temperatures in the turtle in *Emys orbicularis* (Desvages and Pieau, 1992), *Dermochelys coriacea* (Desvages et al., 1993), *Chelydra serpentina* (Rhen and Lang, 1994), and in the slider turtle *Trachemys scripta* (Crews and Bergeron, 1994). Aromatase mRNA is elevated in the putative ovary but not in the putative testis from the diamondback terrapin *Malaclemys terrapin* (Jeyasuria and Place, 1997; Jeyasuria and Place, 1998). In the red-eared slider, aromatase activity was significantly elevated in the brain in females, although there was no difference between sexes for the adrenal-kidney-gonadal axis (Willingham et al., 2000b). Further, treatment with aromatase inhibitors indicates that aromatase is a key enzyme involved in sex determination in many reptiles (Jeyasuria et al., 1994; Richard-Mercier et al., 1995; Rhen and Lang, 1994; Crews and Bergeron, 1994). Estradiol modulates the regulation of steroidogenic factor 1 during gonadogenesis in turtles (Fleming and Crews, 2001).

Other environmental factors besides temperature play a role in the sexual determination of reptiles and these may further obscure cause and effect relationships between potential EDCs and alterations of endocrine function. These factors include alterations in the hydric environment (Gutzke and Paukstis, 1983), CO_2 levels (Jeyasuria and Place, 1998), and higher temperatures that lead to a decrease in pH within the egg. Since 5α-reductase has a strong pH dependence curve, temperature may act indirectly on sexual differentiation by altering pH levels, which subsequently affects the activities of key steroid metabolizing enzymes (Etchberger et al., 1992).

The feeding strategies of reptiles may increase their capacity to bioaccumulate environmental toxins. Reptilian feeding ecology ranges from herbivory to carnivory, with some of the carnivorous reptiles being at or near the top of the food web. Many reptiles, such as turtles, crocodilians, and large snakes, are long lived with life expectancies in the wild that surpass 30 years (Woodbury and Hardy, 1948; Bowler, 1977; Gibbons and Semlitsch, 1982; Congdon et al., 1983). This provides time for significant bioaccumulation of environmental contaminants in their tissues. In fact, reptiles have been shown to bioaccumulate and biomagnify contaminants to levels equal to or greater than birds and mammals (Olafsson et al., 1983; Hall and Henry, 1992; Cobb and Wood, 1997).

4.3.2 Effect based responses and case studies

4.3.2.1 Developmental abnormalities in Lake Apopka alligators

Alligators (*Alligator mississippiensis*) on Lake Apopka, Florida, U.S.A., provide one of the most publicized examples of EDC effects on a wildlife population. In 1980, a stream that feeds Lake Apopka was contaminated with high concentrations of dicofol (including its metabolites DDD, DDE, and chloro-DDT) and other compounds after a chemical spill. Shortly thereafter (1980-1984), the population of alligators declined by 90% (Guillette et al., 1994). A decline in clutch viability was not seen until 1984. Lake Apopka alligators have elevated concentrations of p,p'DDE, dieldrin, endrin, mirex, oxychlordane, DDT, and PCBs (Guillette et al., 1999a). This depressed clutch viability continues today, leading to a depressed population of adult alligators.

Juvenile alligators from Lake Apopka showed a variety of developmental abnormalities (e.g., abnormal gonadal morphology, altered gonadal steroidogenesis, and changes in sex steroid concentrations in males and females), which have been attributed to contaminants that disrupt endocrine function . The evaluations of these endpoints were made in comparison to alligators at a relatively uncontaminated site, Lake Woodruff, Florida. Specifically, the male and female juvenile alligators from Lake Apopka had depressed plasma testosterone levels and elevated 17α-estradiol levels, respectively (Guillette et al., 1994; Guillette et al., 1999a). Also, there were alterations in the steroid (i.e., 17β-estradiol, testosterone) biosynthetic capacity of ovarian and testicular tissue *in vitro* (Guillette et al., 1995; Crain et al., 1997). Typically, hormone concentrations in developing organisms are related to the developmental stage or the size of the organism. As expected, steroid and thyroid hormone levels were related to body size in juvenile alligators from Lake Woodruff, but not in juvenile alligators from Lake Apopka (Crain et al., 1998).

Alligators at Lake Apopka showed a suite of gonadal deformities that were correlated with abnormal plasma steroid levels (Guillette et al., 1994; Guillette et al., 1996). In females, some follicles were found to be polyovular (consisting of three to four oocytes) and selected oocytes were polynuclear (possessing two or three nuclei each) (Guillette et al., 1994). The testes of male alligators exhibited poorly organized seminiferous tubules and many were lined with a cuboidal epithelium. In addition, alterations to sperm cells were identifiable by the presence of elongated, bar shaped nuclei (Guillette et al., 1994). Male alligators showed a significantly reduced phallus size that, at Lake Apopka, varied between areas with different levels of pollution (Guillette et al., 1996; see Figure 4.2). It is unclear whether the male and female alligators exhibiting these gonadal deformities could be sexually potent.

Further research indicates that alterations in steroid hormone concentrations and phallus size are widespread in Florida lakes (Guillette et al., 1999b).

[insert Figure 4.2]

Although the principal contaminant (p,p'-DDE) in eggs and juvenile alligators at Lake Apopka has been identified (Vos et al., 2000), the specific chemical(s) responsible for the observed effects is unknown (Ankley and Giesy, 1998). Several hypotheses have been proposed to explain the contaminant-induced endocrine disruption. For example, multiple EDCs may interact with the alligator estrogen receptor and p,p'-DDE could act as an androgen antagonist in embryonic and juvenile alligators (Guillette et al., 1996; Crain and Guillette, 1998). In addition, the contaminant-induced mechanism of endocrine disruption may involve alterations in aromatase enzyme activity and disruptions in the thyroid-gonad axis (Crain et al., 1997; Crain et al., 1998).

4.3.2.2 Developmental abnormalities in Great Lakes snapping turtles

The developmental abnormalities observed in common snapping turtles (*Chelydra serpentinia serpentinia*) in the Great Lakes- St. Lawrence River basin (Bishop et al., 1998) offer another possible example of the effects of EDCs in reptiles. A report by Bishop et al. (1991) showed that unhatched eggs and hatchling deformities in the snapping turtles occurred from sites with the highest concentrations of chlorinated hydrocarbons during 1986-1988. Bishop et al., (1998) determined that, of the numerous chemicals identified, concentrations of PCBs, PCDDs, and PCDFs in eggs during 1989-1991 were significantly related to the occurrence of abnormalities in developing turtles (Bishop et al., 1998). Several hatchling abnormalities were identified including absent or altered tails, carapace anomalies (missing or extra scutes), unresorbed yolk sacs, and fore and hind limb deformities. Also, it has been suggested that EDCs may affect sexually dimorphic morphology of adult snapping turtles. In snapping turtles, the ratio of the precloacal length to the posterior lobe of the plastron (PPR) is sexually dimorphic. A report by de Solla et al. (1998) found that adult turtles (*Chelydra serpentina serpentina*) from sites contaminanted with OCs exhibited significant reductions in PPR compared with reference site turtles. The same turtles did not show alterations in the levels of sex steroids (i.e., 17β-estradiol and testosterone). Controlled studies of the effects of polychlorinated hydrocarbons on the endocrine physiology (and reproductive fitness) of turtles are needed to establish cause and effect relationships.

4.3.3 Conclusion

Reptiles have received little attention from an ecotoxicological perspective. It is clear that some developmental processes in reptiles, particularly sex determination, gonadal development, steroid hormone synthesis, and development of secondary sex characteristics, are susceptible to endocrine disruption. Although some reptile populations have been impacted by environmental contaminants with endocrine-disrupting properties, it is unclear how widespread the phenomenon is. Currently, there is insufficient data to evaluate whether aquatic reptiles are at greater risk of endocrine disruption compared with terrestrial reptile species for which we have limited data.

4.4 Amphibians

4.4.1 Unique aspects

Metamorphosis occurs almost universally in Amphibia, and is exhibited in the vertebrate groups Agnatha (jawless fishes) and Osteichthyes (bony fishes), as well. The remaining vertebrates (Chondrichthyes (cartilaginous fishes), reptiles, birds, and mammals) do not exhibit metamorphosis (Norris, 1983). The life histories of extant amphibians are diverse, with some species experiencing complex transformations that progress from free-swimming aqueous life-stages to terrestrialness. These transformations involve a myriad of structural and biochemical changes to processes such as respiration, osmoregulation, waste (nitrogen) excretion, and locomotion. Therefore, this class of vertebrates may be subject to EDC exposures during different stages of their life cycle and may be particularly at risk of the effects of EDCs (Vos et al., 2000). The endocrine regulation of metamorphosis is comprised of several developmental hormones that could be affected by EDCs, particularly the thyroid hormones (T3/T4), but also, corticosteroids, prolactin, and retinoic acid derivatives (Norris, 1996). It has been postulated that water soluble and non-persistent chemicals may affect metamorphosis. It is unlikely that these putative EDCs would be identified in traditional ecotoxicological assays, which concentrate on persistent, bioaccumulative chemicals. Currently, there is insufficient data to discern whether or not metamorphosis in amphibians is especially sensitive to the effects of aquatic contaminants with endocrine disrupting properties.

Amphibians may be exposed to EDCs via several different routes because of their semipermeable skin, the development of their eggs and gill breathing larvae in the water, and their position in the food web, which changes from herbivory of tadpoles to carnivorous adults (Gutleb et al., 1999). Hibernation is another life history trait of many amphibians during which time species remain submerged in the

substrate, which may make them more vulnerable to toxins. It has been suggested that amphibians could be more susceptible to the effects of environmental contaminants during hibernation (Russell et al., 1995), as the same doses of DDT have been found to be only moderately toxic to adult common frogs (*Rana temporaria* L.) in oral doses, but results in mortality under conditions of food deprivation (Harri et al., 1979).

4.4.2 Effect based responses and case studies

4.4.2.1 Amphibian population changes

Currently, there is little dispute within the scientific community that adverse population-level effects in amphibians are occurring (e.g., Pechmann et al., 1991; Pechmann and Wilbur, 1994). Populations are declining in both pristine and polluted habitats worldwide (Vos et al., 2000) and there have been several recent reviews that describe the amphibian populations that are affected (e.g., Sarkar, 1996; Green, 1997a, b; Lannoo, 1998; Corn, 1999). A variety of changes in amphibian populations have been noted, including species extinctions, decreases in population size, and changes in the spatial distribution of certain species. Primarily, efforts have concentrated on alterations of amphibian populations in North and Central America, as well as in Australia and Europe (Corn, 1999). Comparatively little is known concerning the status of amphibian populations in Asia and Africa.

A study by Carey and Bryant (1995) examined the potential for environmental contaminants to affect amphibian population numbers through the disruption of growth and development of the young. Their conclusion was that critical data are lacking in most documented cases, which precluded drawing a link between altered populations and chemical exposures. Currently, there is little evidence to support the hypothesis that the effects observed in amphibians in the field are the result of EDCs. An exception is the report by Kirk (1988) that showed a strong association between DDT spraying and effects on a local population of Western spotted frogs (*Rana pretiosa*). Several other reports are more speculative (e.g., Drost and Fellers, 1996; McConnell et al., 1998). For example, in 1993, Russell et al. (1995) examined a population of spring peepers (*Pseudacris crucifer*) at Point Pelee National Park, Canada, and found that these frogs had appreciable levels of DDT, DDE, DDD, and dieldrin. This locality was the site of frequent DDT spraying until 1967. Since 1972, cricket (*Acris crepitans*), gray tree (*Hyla versicolor*), and bull (*Rana catesbeiana*) frogs have become locally extinct.Researchers have not been able to attribute the declines in amphibian populations to obvious environmental factors (i.e., habitats have actually improved and acid rain has been ruled out). The authors suggested that the application of pesticides decades ago might have been an important factor in the decline of amphibians at this park. In these examples, the

mechanism of action of the putative agents is uncertain and it is unknown whether the mechanism was endocrine-mediated.

There is limited evidence from controlled laboratory studies that amphibians are affected by known EDCs in a manner consistent with other vertebrate species (e.g., Hall and Henry, 1992). For example, vitellogenesis is induced in African clawed frogs (*Xenopus laevis*) by exposure to o,p-DDT, dieldrin, and toxaphene (Palmer and Palmer, 1995; Palmer et al., 1997). As discussed by Carey and Bryant (1995), EDCs may affect amphibians either directly (e.g., alterations in developmental hormones), or indirectly (e.g., increased disease susceptibility associated with compromised immunity).

4.4.2.2 Deformities in amphibians

There have been several recent reports of deformed frogs in abnormally large numbers throughout North America (Ouellet et al., 1997; Schmidt, 1997; Ankley and Giesy, 1998). Most commonly, the affected species appear to be Ranids, such as the northern leopard frog (*Rana pipiens*), green frogs (*Rana clamitans*), and the mink frog (*Rana septentironalis)*. The predominant malformations include missing or supernumerary limbs, bony limb-like projections, digit and musculature malformities, as well as eye and central nervous system abnormalities (Ankley and Giesy, 1998). It is unknown whether the instances of population declines can be attributed to the deformities, and/or whether the same environmental factor(s) is responsible for both of these effects.

Despite the absence of a cause and effect relationship between EDCs and amphibian deformities, there is a strong mechanistic basis for a chemical-based etiology of amphibian malformations. It has been postulated that environmental retinoids, or "retinoid mimics", may contribute to the induction of developmental effects of ranids in the wild (Ankley and Giesy, 1998). In addition to thyroid hormones, early embryonic development in amphibians is strongly dependent on the retinoic acid (RA) hormonal system comprised of active retinoids (i.e., all-trans-retinoic acid, 9-cis-retinoic acid, and structurally related derivatives) and a number of retinoid receptors (Schena, 1989). The RA system controls different developmental processes including pattern formation and limb development (Wagner et al., 1990; Shimeld, 1996). RA treatment during embryogenesis can result in developmental abnormalities as was shown in case of *Xenopus laevis* embryos (Papalopulu et al., 1991) and limb defects have been reported in a variety of other vertebrate models (Maden, 1993; Rutledge et al., 1994; Scott et al., 1994). A recent study by Gutleb et al. (1999) reported that a non-ortho PCB congener (PCB 126) caused dose-related malformations (oedema, lack of gut coiling, misformed eyes and tails) in *X. laevis* embryos and that

retinoid concentrations were significantly altered in PCB dosed embryos. A metabolite of the environmental contaminant, methoprene, has been shown in transfected cell lines to activate a retinoic acid receptor (Harmon et al., 1995), which has prompted speculation that this pesticide could be involved in the production of limb malformations in native anurans. However, studies are required to validate this hypothesis. Further research is needed to elucidate whether or not environmental contaminants are responsible for the observed malformations in amphibians, and if so, whether or not these effects are mediated through endocrine disrupting mechanisms of action.

Other factors besides chemical stressors need to be considered as possible causative agents of amphibian malformations. Amphibian limb malformations may be due to the physical disruption of limb buds by trematode cysts (Sessions and Ruth, 1990). Certain trematodes, which utilize larval amphibians as a secondary host, can occur at relatively large concentrations as metacercaria in the developing limb bud field. A recent study showed that severe limb abnormalities were induced in Pacific tree frogs (*Hyla regilla*) exposed to cercariae of a trematode parasite (Johnson et al., 1999). These abnormalities closely matched those observed at field sites. In addition, it has been postulated that increased UV exposure, associated with stratospheric ozone depletion, may be responsible for some amphibian malformations (UNEP, 1998). Controlled experiments have shown that UV light, as well as natural sunlight, can cause hindlimb malformations (ectromelia and ectrodactyly) in the northern leopard frog (Ankley et al., 1998b; Ankley et al., 1999).

4.4.3 Conclusion

Currently, there are insufficient data to implicate EDCs as causative agents in amphibian declines. Also, there is not enough conclusive evidence to state that environmental contaminants are responsible for the observed malformations. Clearly, further work is needed to assess this possibility. It is worthy to note, however, that increasingly more studies are focusing on amphibians as potential target species of EDCs (e.g,., Harris et al., 1998a; Harris et al., 1998b).

4.5 Fish

4.5.1 Unique aspects

Fish are the most successful group of vertebrates and display a high degree of heterogeneity in their physiology, anatomy, behaviour, and ecology. There are over 3,000 species of cartilaginous fish (e.g., elasmobranchs and chimaeras), over 20,000 species of bony fish (e.g., teleosts, dipnoans, and holosteans), and fewer species of the ancient jawless fish (e.g., lampreys and hagfish). Only a small

proportion of fish species has been thoroughly investigated and data on cyprinids and salmonids (both teleosts) dominate the literature.

Fish have evolved to inhabit a range of aquatic environments (e.g., freshwater, brackish, and marine) that differ in osmotic properties. Both aquatic respiration and osmoregulation may contribute to an increased exposure of EDCs to fish. The higher ventilation rate of fish compared to humans may increase their exposure to waterborne contaminants to the respiratory surfaces of the gills (Van Der Kraak et al., 2001). In addition, other features of fish gills (e.g., countercurrent system of blood and water flow, thin epithelial membranes, and a high surface area) may increase the uptake of compounds from the water and their transfer to the bloodstream. Marine teleosts drink seawater due to their hypotonic nature, which may contribute to their exposure to waterborne substances. In contrast, hypoosmotic freshwater fish (these fish do not drink) move water into their bodies thereby creating a route of exposure to waterborne contaminants.

A high degree of diversity is displayed in the reproductive strategies of fish (Kime, 1998). The majority of fish are oviparous, but species displaying ovoviviparity and true viviparity are also widespread. Of the oviparous species, reproductive strategies range from the production of many small eggs (e.g., up to 28 million in a single cod) that are released into the plankton, to fewer and larger demersal eggs, which may be laid in a nest and guarded. In addition, some elasmobranchs have very few large, yolky eggs. A significant route of exposure for early life-stage fish is through the maternal transfer of hydrophobic xenobiotic contaminants sequestered in lipid reserves. This route of exposure may be greater than the levels accumulated directly from the water column. As well, the limited activity of biotransformation and excretory enzymes of early life-stages may maximize exposure during critical periods of development (Van Der Kraak et al., 2001).

Sexual plasticity is another reproductive strategy in certain species of fish. Although fish are usually gonochoristic (separate sexes), functional hermaphrodites do exist (e.g., Serranidae and Sparidae), which is uncommon among vertebrates (Chan and Yeung, 1983). In some species of teleost, particularly coral reef fishes, individuals can reversibly change from one functional sex to the other in response to environmental (e.g., temperature) and social cues (e.g., pheromones) (Stahlschmidt-Allner and Reinboth, 1991). It is unknown how the sexual plasticity that occurs in some species of fish may be affected by environmental contaminants with endocrine disrupting properties. However, the existence of this natural sex change implies that irreversible sexual imprinting does not occur in fish in the same manner as it does in the brain of fetal mammals.

4.5.2 Effects based responses and case studies

4.5.2.1 Induction of vitellogenesis in juvenile or male fish

The induction of vitellogenin (VTG) production in juvenile or male fish in the field has become one of the most notable and convincing biological responses of fish linked to EDC (i.e., estrogenic compounds) exposure (Tyler and Routledge, 1998; Kime et al., 1999). Currently, there are numerous cases of VTG induction in fish in a variety of water bodies across Europe, Japan, and North America. The most dramatic increases in VTG levels appear to be in fish exposed to sewage treatment works (STW) discharges at sites in the United Kingdom. The implications of these responses in both freshwater and marine fish in terms of reproductive success and viability are unknown.

Research by Purdom et al. (1994) found that male rainbow trout (*Oncorhynchus mykiss*) caged in STW discharges in England had elevated plasma VTG levels (up to 147 mg/ml) that were equal to or exceeded the levels found in mature females. Caged immature carp (*Cyprinus carpio*) also showed elevated VTG levels, but to a lesser extent. Subsequent work by Harries et al. (1996; 1997) showed that several rivers in the United Kingdom also contained sufficiently high concentrations of estrogenic compounds to induce vitellogenesis in male rainbow trout. In addition to VTG induction, fish exposed downstream of discharges sometimes exhibited enlarged livers and reduced testicular growth. Typically, the degree of vitellogenesis declined rapidly with distance from the source of pollution and much of this was attributed to dilution of the active compounds (Harries et al., 1999). Also, induction of plasma VTG in chub (*Leuciscus cephalus*) has been observed in the River Moselle in France downstream of urban areas (Flammarion et al., 2000).

There are several more examples of VTG induction in freshwater fish exposed to municipal sewage discharges. Different localities in the U.S.A. revealed similar responses in male carp (Bevans et al., 1996; Folmar et al., 1996; Goodbred et al., 1997) and fathead minnows (*Pimephales promelas*) (Nichols et al. 1999) exposed to municipal wastewaters. In Sweden, the bile of juvenile rainbow trout caged in STW discharge for two weeks contained the natural estrogens, 17β-estradiol and estrone, and the synthetic estrogen, 17α-ethinylestradiol, and plasma VTG levels were high (1.5 mg/ml) (Larsson et al., 1999).

Primarily, VTG inductions in the documented cases discussed above have been attributed to environmental estrogens that are either synthetic or natural compounds. For example, in the River Aire,

nonylphenol (NP) and its ethoxylates, which were previously discharged into the water in large amounts, have been implicated as causative agents in the VTG responses of the fish. In controlled laboratory studies with rainbow trout, Jobling et al. (1996) demonstrated that NP, at environmentally relevant concentrations (as low as 20 μg/L), was able to induce VTG production and retard testicular growth. The natural (i.e., 17β-estradiol, estrone) and synthetic (i.e., ethynylestradiol) estrogen hormones may contribute substantially to the observed responses (Routledge et al., 1998). These hormones enter the sewage as inactive conjugates, but can be de-conjugated into active compounds by bacterial enzymic activity (Panter et al., 1999). As a result of measuring a number of estrogenic compounds in fish bile, another estrogenic compound, bisphenol-A has also been added to the list of probable causative agents (Larsson et al., 1999).

The induction of VTG is not confined to only freshwater fishes. VTG induction and testicular abnormalities were detected in male European flounder (*Platichthys flesus*) that were caught near a STW discharge in the Tyne estuary, United Kingdom (Lye et al., 1997; Lye et al., 1998). Later work partly associated these effects with exposure to, and bioaccumulation of, several estrogenic alkylphenols (Lye et al., 1999). Subsequent investigations in Britain (Matthiessen et al., 1998; Allen et al., 1999a; Allen et al., 1999b) have measured high levels of VTG (up to 20 mg/ml plasma) in male flounder from heavily industrialized estuaries (i.e., Tyne, Tees, Wear, and Mersey estuaries; see Figure 4.3). In contrast, low or undetectable levels of VTG were found in male flounder from estuaries draining rural areas or urban areas with relatively little heavy industrial activity. Similar effects to those described in flounder from the United Kingdom have also been observed in Japanese flounder (*Pleuronectes yokohamae*) from Tokyo Bay (Hashimoto et al., 2000). The causative substances have not yet been fully identified, but as with freshwaters, mixtures of natural and synthetic estrogenic substances have been detected in estuarine waters. However, most estuarine estrogenic activity appears to be adsorbed on the sediment solid phase, yet the majority of this activity remains to be identified (Thomas et al., 2001).

[insert Figure 4.3]

Pulp and paper mill effluents may also contain estrogenic compounds that alter VTG production in exposed fish. In one of three mills studied in Finland, VTG gene expression was induced in caged whitefish (*Coregonus lavaretus* L.) held for 4 weeks in the effluent (Mellanen et al., 1999). This mill was found to discharge considerably more wood-derived compounds (e.g., sterols, resin acids) than the other two mills. Pelissero et al. (1991a; 1991b) demonstrated that VTG was produced in male and juvenile sturgeon (*Acipenser baeri*) exposed in the laboratory to a variety of phytoestrogens. In addition,

controlled laboratory studies by Tremblay and Van Der Kraak (1998; 1999) demonstrated that exposure of sexually immature rainbow trout to either bleached kraft mill effluent (BKME) or to β-sitosterol (a phytosterol found in the effluent) for three weeks results in the induction of vitellogenesis. However, field studies of feral white sucker (*Catostomus commersoni*) downstream of BKME discharge in Canadian waters have failed to show VTG induction (Van Der Kraak et al., 1998b).

4.5.2.2 Reproductive abnormalities

4.5.2.2.1 Altered gonadal development induced by sewage treatment works (STW) discharges

The first documented cases of abnormal gonadal development in fish were those found to occur concomitantly with VTG induction discussed in the previous section. Again, a large proportion of the data has been generated from studies of fish exposed to STW discharges in the United Kingdom. These fish are indisputably affected by contaminants with estrogenic activity. The inhibition of testicular growth has been observed in adult rainbow trout held in waters heavily contaminated with estrogenic compounds such as in the River Aire, which is contaminated with alkylphenols (Harries et al., 1997). Trout exposed in laboratory studies to environmentally realistic concentrations of alkylphenols display similar effects on testicular growth (Jobling et al., 1996). Also, occurrences of ovotestis have been documented in wild male roach (*Rutilus rutilus*) and gudgeon (*Gobio gobio*) collected downstream of STW discharges in several rivers in the United Kingdom. The proportion of fish found with ovotestis varies from just a few percent to 100% in the case of wild roach in some rivers (e.g., Aire and Nene).

The severity of the ovotestis condition ranges from the occasional oocyte in otherwise normal testicular tissue, to large regions of mature ovarian tissue interspersed with abnormal testicular tissue. Presumably this condition is caused by exposure of the fish to estrogenic compounds during critical stages of gonadogenesis (Jobling et al., 1998a; Tyler and Routledge, 1998). Other testicular abnormalities documented in some of the fish exposed to STW discharges include feminized or absent vas deferens and impaired milt production (Jobling et al., 1998b). Feminization of the vas deferens has been described in all-male carp larvae exposed in the laboratory to 4-*tert*-pentylphenol (Gimeno et al., 1996; Gimeno, 1997; Gimeno et al., 1998a, b).

Wild European flounder from heavily industrialized estuaries (the Mersey and Tyne) and a bay (Seine) were reported to have altered spermatogenesis (Lye et al., 1998) and up to 18% showed ovotestis (Matthiessen et al., 1998; Allen et al., 1999a; Allen et al., 1999b, Minier et al., 2000; see Figure 4.4). However, most estuaries do not contain any flounder with ovotestis. It has been hypothesized by

Matthiessen et al. (1998) that flounder do not show higher prevalences of ovotestis (in estuaries where VTG is nevertheless induced) because their larvae probably undergo gonadogenesis while still at sea, under relatively uncontaminated conditions. Although the causes of these effects in UK estuaries are unknown, circumstantial evidence suggests that endocrine disruption from natural and synthetic estrogenic compounds may be responsible (Lye et al., 1999; Thomas et al., 2001). Ovotestis has also been observed in organochlorine-contaminated shovelnose sturgeon (*Scaphirhynchus platyorynchus*) living in the Mississippi River downstream of St. Louis, Missouri (Harshbarger et al., 2000). Finally, Batty and Lim (1999) have shown that male mosquitofish (*Gambusia affinis*) living downstream of a sewage treatment plant in Australia possess gonopodia of reduced size. The gonopodium is an anal fin modified during sexual development for the deposition of sperm, but it is unknown if reduced size affects fertilization success.

4.5.2.2.2 Reproductive abnormalities induced by pulp and paper mill effluents

Reproductive abnormalities have been reported in fish exposed to pulp and paper mill effluents. Primarily, field studies have focused on the effects of effluents on fish (e.g., white sucker, longnose sucker *Catostomus catostomus*, lake whitefish *Coregonus clupeaformis*) in Canadian waters (e.g., Munkittrick et al., 1991; Gagnon et al., 1995; Van Der Kraak et al., 1998b; Munkittrick et al., 1998), but similar effects have been reported in fish (e.g., Eurasian perch *Perca fluviatilis*, blenny *Zoarces viviparus*) in Scandinavia (e.g., Andersson et al., 1988; Neuman and Karås, 1988; Sandström et al., 1988; Larsson et al., 1997).

In Canada, white sucker collected downstream of BKME outfalls at Jackfish Bay, Lake Superior, exhibited a wide array of altered reproductive responses, including reductions in gonad size, delayed sexual maturity, and reduced expression of secondary sexual characteristics (decreased nuptial tubercles on males) compared with control sites (Van Der Kraak et al., 1998b; see Figure 4.5). The viability of eggs and sperm and the viability of developing larvae of these suckers appeared to be normal (McMaster et al., 1992). Similar reproductive effects have been observed in laboratory studies using fathead minnows exposed to BKME (Kovacs et al., 1995). Lake whitefish collected at Jackfish Bay also exhibited reproductive abnormalities and delayed sexual maturation (Munkittrick et al., 1992a, b). In perch, similar effects (reduced gonad size and delayed sexual maturity) have been observed, coupled with impaired fry production, smaller embryos, increased larval mortality and reduced abundance (Larsson et al., 1997). Effects observed downstream of pulp and paper mill discharges tend to decrease with

increasing distance from the outfalls. In most cases, there is sufficient evidence to conclude that the effluents are responsible for the observed effects.

[insert Figure 4.5]

The active compounds responsible for the reproductive abnormalities in BKME-exposed fish have not been identified. However, several compounds may contribute to the effects including β-sitosterol, other sterols, lignans, stilbenes and resin acids, which are compounds with weak estrogenic activity (Adlerkreutz, 1988; Mellanen et al., 1996). Other possible compounds are stigmastanol and a β-sitosterol degradation product, which have androgenic properties (Rosa-Molinar and Williams, 1984; Howell and Denton, 1989). Furthermore, pulp and paper mill effluents can exert both estrogenic and anti-estrogenic activity in mammalian cells *in vitro* (Zacharewski et al., 1995). As well, the livers of BKME-exposed white sucker rapidly accumulate ligands for the estrogen receptor, androgen receptor, and sex steroid binding protein (Hewitt et al., 2000). Although the situation is complex, the reproductive effects and hormonal changes indicate that constituents of pulp and paper mill effluents disrupt endocrine function in of exposed fish.

Contrasting effects of BKME have been observed on the secondary sexual characteristics of female mosquitofish (*Gambusia affinis*) in streams below paper mills in Florida, U.S.A. These fish showed strong masculinization of the anal fin, which developed into a male-like gonopodium (Howell et al., 1980; Bortone and Cody, 1999). In males, the development of the anal fin into a gonopodium is under androgenic control. Therefore, it was suggested that the female mosquitofish had been exposed to androgenic compounds found in the effluent. Recent studies by Parks et al. (2001) determined that pulp mill effluent from a Florida mill contained compounds that exhibited androgen agonistic activity at levels sufficient to account for the masculinization of female mosquitofish. It is not known whether the differential effects of the BKME on fish in Florida and Canada are due to differences in species sensitivities or to different substances discharged in the BKME.

The mechanism(s) behind the effects of BKME on secondary sexual characteristics is not well understood. However, it is plausible that the demasculinizing effect of BKME on feral fish in Jackfish Bay may be due to the concomitant decrease in plasma androgen (e.g., 11-ketotestosterone) levels (Munkittrick et al., 1991). It is possible that the masculinizing effect of the effluent may involve the actions of phytosterols. Denton et al. (1985) and Howell and Denton (1989) showed that partly biodegraded mixtures of plant sterols (ß-sitosterol, campestrol and stigmastanol) were able to induce non-

reversible masculinization of the anal fin in adult female mosquitofish, but that undegraded sterols were inactive. The precise mechanisms of action of these degraded sterols are poorly understood but the production of androstane-like compounds may interfere at a site within the pituitary-gonadal axis, or act agonistically at the androgen receptor. The latter mode of action has been observed in adult female goldfish (*Carassius auratus*), which develop male secondary sexual tubercles after exposure to implanted 11-ketotestosterone (Kobayashi et al., 1991).

4.5.2.2.3 Possible cases of reduced reproductive success

There are very few examples of wildlife being impacted at the population level by exposure to EDCs. In fish, one of the best examples of possible effects of EDCs on reproductive success in the field involves the PAH and PCB contaminated areas of Puget Sound on the west coast of the U.S.A. (Collier et al., 1998). A series of elegant studies on several flatfish populations including rock sole (*Pleuronectes bilineatus*) (Johnson et al., 1998), winter flounder (*Pleuronectes americanus*) (Johnson et al., 1992), and English sole (*Parophrys vetulus*) (Johnson et al., 1988; Casillas et al., 1991; Johnson et al., 1997; Landahl et al., 1997) have demonstrated a range of reproductive effects such as precocious female maturation, inhibited ovarian development, reduced egg weight, reduced spawning success, and reduced larval survival. The precise mechanisms behind these effects are not well understood, but they have been correlated with elevated levels of PAHs, DDT/DDE, and PCBs. The majority of the effects (especially reduced fecundity and spawning success) may be due to the anti-estrogenic effects of some PCBs and PAHs, or the interactions of PAHs or dioxins with the aryl hydrocarbon (AhR) receptor. Stein et al. (1991) demonstrated that English sole injected with extracts of sediments from Puget Sound had reduced plasma E_2 levels. The estrogenic effects of various OCs may contribute to the precocious female maturation observed in English sole (Collier et al., 1998; Johnson et al., 1997). However, non-endocrine modes of action also need consideration due to the large number of contaminants present in the Puget Sound area.

4.5.2.2.4 Less developed case studies

There are several less developed examples suggesting that exposures to putative EDCs are impacting reproductive success in fish. For example, reduced production of perch (*Perca fluviatilis*) fry has been associated with endocrine disrupting discharges of BKME in Sweden (Neuman and Karås, 1988; Sandström et al., 1988). Furthermore, reduced hatching success, reduced embryo and larval survival, and slower rates of development in fry have been reported in Atlantic herring (*Clupea harengus*) (Hansen et

al., 1985), Atlantic cod (*Gadus morhua*) (Petersen et al. 1997), and European flounder (Von Westernhagen et al., 1981) from the Baltic Sea, lake trout (*Salvelinus namaycush*) from the Great Lakes (Mac and Edsall, 1991; Mac et al., 1993), and Arctic char (*Salvelinus alpinus*) from Lake Geneva (Monod, 1985).

Cases of possible but unconfirmed endocrine disruption-related impacts on gonad development by potential EDCs are numerous, and include reduced and delayed ovarian development in flatfish after the *Amoco Cadiz* oil spill in Brittany (Stott et al., 1983), decreased egg weight and increased atresia in flatfish from contaminated harbours in the eastern USA (Johnson et al., 1992), and premature maturation in flatfish from the southern North Sea (Rijnsdorp and Vethaak, 1997) and Puget Sound (Collier et al., 1998, Johnson et al., 1997). These examples could be due to a range of possible endocrine-mediated mechanisms (including receptor-mediated effects, effects on the pituitary-gonadal pathway, or interference with steroid metabolism), yet, non-endocrine toxicity, or other environmental factors cannot be ruled out.

4.5.2.3 Altered sex steroid levels

In addition to the effects described above, there are several reports of reduced levels of sex steroids in both male and female fish exposed to phytoestrogens and BKME. For example, female white sucker exposed to BKME during vitellogenesis (period of gonadal development) in Jackfish Bay, Lake Superior, showed reduced levels of circulating 17β-estradiol (E_2) and testosterone (T) (Munkittrick et al., 1991; McMaster et al., 1991; Munkittrick et al., 1994), and reduced T and 17,20) β-dihydroxy-4-pregnen-3-one (17,20, β) during the prespawning and spawning period (McMaster et al., 1991; Van Der Kraak et al., 1992) compared with levels of steroids found in female white suckers at reference locations. Similar depressions of 11-ketotestosterone (11-KT), T, and 17,20,β were found in male white suckers. Also, longnose sucker and lake whitefish collected in the vicinity of Jackfish Bay (Munkittrick et al., 1992a, b), other fish species from additional BKME-exposed sites in North America (e.g., Hodson et al., 1992; Gagnon et al., 1994; Adams et al., 1992), and perch and roach exposed to pulp mill effluents in Sweden and Finland have been reported to have depressed hormonal levels (Larsson et al., 1997; Van Der Kraak et al., 1997; Karels et al., 1999). A similar syndrome has been seen in BKME-exposed mummichogs (*Fundulus heteroclitus*) (Dubé and MacLatchy, 2000). In laboratory experiments, fathead minnows exposed to BKME over their life cycle exhibit a parallel range of effects on circulating steroids and reproductive endpoints (e.g., Robinson, 1994; Kovacs et al., 1995). Together, these studies provide

evidence that the reproductive responses were associated directly with effluent exposure and were not the result of other environmental factors such as habitat alteration.

Multiple locations in the hypothalamus-pituitary-gonad axis appear to be affected by constituents in BKME with endocrine disrupting properties. During their spawning migration in Jackfish Bay, white sucker were found to have altered pituitary function, as determined by the depressed levels of plasma gonadotropin hormone (GtH-II) in males and females that were approximately 30 to 50 fold less than levels detected in control fish from a reference location (Van Der Kraak et al., 1992). In addition, exposed fish had a diminished response to a gonadotropin-releasing hormone (GnRH) analog (as determined by GtH levels) and *in vitro* studies showed that ovarian steroid biosynthetic capacity (i.e., T and 17,20,β) was reduced. Finally, BKME-exposed fish had depressed levels of glucoronidated testosterone, which indicates that peripheral steroid metabolism was altered. Within the steroid biosynthetic pathway of the gonads, the predominant effect of BKME occurs downstream of pregnenolone (the immediate metabolite of cholesterol) (McMaster et al., 1995) and may inhibit the steroidogenic enzyme, 17α-hydroxylase/C_{17-20} lyase.

The constituents of BKME that are responsible for, or contribute to, the endocrine disruption observed in exposed fish have not been conclusively identified. The weakly estrogenic phytosterol, b-sitosterol is one compound known to be present in BKME that has been investigated as a possible EDC. In laboratory studies, goldfish exposed to β-sitosterol exhibit many of the reproductive effects observed in feral fish populations exposed to BKME, such as depressed levels of T, 11-KT, and E_2 (MacLatchy et al., 1997). Other studies have reported that β-sitosterol does not act at the level of the estrogen receptor; rather it interferes with the availability of cholesterol and pregnenolone (possibly via effects on cAMP formation), which impairs the steroid biosynthetic capacity of the gonads (MacLatchy and Van Der Kraak, 1995; Tremblay and Van Der Kraak, 1999). It is obvious that further work is necessary to identify the potential EDCs that are present in BKME and to discern how they elicit their effects on reproductive processes in fish.

Several other examples of altered levels of circulating sex steroids in fish have been noted in the field. For example, Folmar et al. (1996) found the male carp (*Cyprinus carpio*) collected near estrogenic STW discharges in the U.S.A. exhibited reduced levels of T, but not E_2. Elevated E_2/11-KT ratios were reported in wild carp that contained levels of pesticide residues that were above normal (Goodbred et al., 1997). Decreased plasma GtH and E_2 levels, but elevated ovarian T production were correlated with increasing body burdens of organochlorines in female kelp bass (*Paralabrax clathratus*) in the South

California Bight (Spies and Thomas, 1997). Female European flounder held for three years on harbour sediment that was contaminated predominantly with PCBs and PAHs showed elevated T and E_2 levels, which may have been the result of decreased steroid clearance via the cytochrome P450 system (Janssen et al., 1997). Furthermore, male European flounder from estrogen contaminated United Kingdom estuaries contain elevated titres of estradiol (Scott et al., 2000). Of all the examples of altered sex steroid levels in fish from field studies, the case of BKME is the best understood.

4.5.2.4 Altered adrenal physiology

In fish, as in other animals, stress results in the activation of the hypothalamus-pituitary-interrenal (HPI) axis, culminating in increased plasma cortisol concentrations. There is now evidence from field studies that environmental contaminants may chronically stress fish resulting in a compromised HPI response. For example, Hontela et al. (1992; 1995) demonstrated that yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) from Canadian sites contaminated with PAHs, PCBs, and heavy metals were unable to produce cortisol in response to acute handling stress and their adrenocorticotropic hormone (ACTH)-producing cells (i.e., pituitary corticotrophs) were atrophied. It was speculated that the atrophy was the result of prolonged hyperactivity of these cells. Consequences of this might involve an impaired ability of the fish to regulate energy metabolism. It was also suggested that the clearance rate of cortisol might have been increased by the contaminant-induced cytochrome P450 mixed function oxidase system. In both sexes of yellow perch, gonad size and thyroid hormone (T4) levels were reduced. Other studies by Hontela et al. (1997) showed that the effects of BKME were similar to the effects of PCBs, PAHs, and heavy metals. In these studies, both the corticotrophs and the interrenal steroid producing cells were atrophied.

Recent work by Norris et al. (1997b; 1999) showed that brown trout (*Salmo trutta*) living in metal-contaminated waters in the U.S.A. had comparable levels of cortisol compared with control fish (both groups of fish were stressed by electroshocking). Yet, the fish exposed to metals were found to be hypersecreting ACTH and corticotropin-releasing hormone (CRH) to maintain baseline levels of cortisol. Similar studies demonstrated that fish chronically exposed to metal contaminants could not maintain ACTH and cortisol levels compared with controls in acute stress trials (Norris et al., 1999). Another report by Girard et al. (1998) showed that yellow perch from contaminated sites synthesize less cortisol in response to an injection of ACTH compared with fish collected from control sites. This indicates that damaged interrenal tissue contributes to the observed effects, as ACTH could not alleviate the impaired

cortisol response. More research is necessary to determine the extent of EDC-induced compromised stress responses in fish, as well as its potential impacts on the health of populations.

4.5.2.5 Early life stage mortality

Contaminant-induced endocrine disruption may be responsible for blue-sac disease in Great Lakes salmonids, which is a condition characterized by yolk sac edema, regional ischaemia, hemorrhaging, craniofacial abnormalitites, and mortality early in larval development (Symula et al., 1990; Cook et al., 1997; Ankley and Giesy, 1998; see Figure 4.6). Probably the most developed cases involve lake trout, but similar conditions have been reported in several species of *Salvelinus*, *Oncorhynchus*, and *Salmo*. This condition has been reproduced in laboratory studies using TCDD (and related compounds), as well as extracts of adult lake trout from Lake Michigan, which contained PCDDs, PCDF, and PCB congeners (Giesy and Snyder, 1998). A retrospective analysis of the incidences of blue-sac disease and exposure to these contaminants found a relationship between observed trends in lake trout reproduction in Lake Ontario. It concluded that, in the past, Ah receptor agonists were primary contributors to the adverse population level impacts in this system. However, possible mechanistic linkages between Ah receptor agonists and endocrine disruption are poorly understood.

Another condition noted in 1968 to the present in Great Lakes fish was Early Mortality Syndrome (EMS) (Marcquenski and Brown, 1997). This syndrome affects the fry and is characterized by a loss of equilibrium, spiral swimming, lethargy, hemorrhage, and death. An analogous condition occurs in Atlantic salmon (*Salmo salar*) inhabiting the Baltic Sea and is termed M74 (Börjeson and Norrgren, 1997; Bengtsson et al., 1999). While the exact cause of EMS and M74 in the wild is unknown, diet may play a role. Laboratory experiments have shown that both EMS and M74 are caused by thiamine deficiency and could be related to differential thiamine or thiaminase contents in prey species.

There are data suggesting that contaminants may play a role etiology of EMS and M74. For example, Tillitt et al. (1996) showed that thiamine can protect against the deleterious effects of dioxin to fish embryonic development, so a natural thiamine deficiency could exacerbate the effects of OC contamination. Also, there seems to be a correlation between incidences of M74 and elevated body burdens of PCDFs and coplanar PCBs (Vuorinen et al., 1997a; Vuorinen and Keinänen, 1999). Another mechanism that has been suggested is that M74 may be the result of toxicant-induced alterations in thyroid hormone and retinol levels, but the evidence is weak (Vuorinen et al., 1997b). Both syndromes

have not been conclusively linked to exposure to EDCs, nor has an endocrine-based mechanism been demonstrated.

The deaths of juvenile salmon in Canadian river basins exposed to the insecticide, Matacil 1.8D, provides another example of possible endocrine disruption in feral fish. Matacil 1.8D is formulated with nonylphenol (NP). Fairchild et al. (1999) showed that a significant proportion of the lowest Atlantic salmon catches, and heavy salmon smolt mortality, coincided with spraying of Matacil 1.8D for spruce budworm control. A similar product that is formulated without NP (Matacil 1.8F) did not have these effects. Low catches of blueback herring (*Alosa aestivalis*) also coincided with the spraying of Matacil 1.8D. Fairchild et al. (1999) estimated that the spraying of the insecticide would have resulted in sufficient concentrations of NP in the river water to cause estrogenic effects in the fish. Furthermore, Madsen et al. (1997) demonstrated in the laboratory that NP could inhibit smoltification and impair hypo-osmoregulation in salmon. It is known that sex steroids and the entire process of sexual maturation antagonize the physiological processes of smoltification and seawater acclimation. However, the precise mechanisms involved, and the actions of NP on these processes are not well understood.

4.5.2.6 Thyroid dysfunction

Salmonid species in the Great Lakes have been found to suffer from abnormally high incidence of thyroid dysfunctions (i.e., goitre). Although enlargement of the thyroid gland in vertebrates can be due to a natural iodine deficiency in the diet, this has been ruled out as a causative factor in the case of these salmonids (Leatherland 1994). Depressed thyroid hormone levels have been observed in many salmonid species in the Great Lakes, particularly Lake Michigan and Lake Erie (e.g., Leatherland and Sonstegard, 1980; Leatherland and Sonstegard, 1984; Leatherland et al., 1989). It was originally suspected that OCs, functioning as environmental goitrogens, might be responsible. Laboratory studies of salmon chronically dosed with PCBs or perchlordecone did exhibit lowered thyroid hormone levels (Leatherland and Sonstegard, 1978). However, feeding trials in which rainbow trout and coho salmon (*Oncorhynchus kisutch*) were fed OC-contaminated fish from the Great Lakes failed to produce the same effects (Leatherland, 1992; Leatherland, 1993; Leatherland and Sonstegard, 1982). Nevertheless, goiters and depressed serum thyroid hormone levels were produced in rodents fed contaminated fish (Leatherland, 1992; Leatherland, 1993), indicating the presence of unidentified goitrogens. It is still not known which compounds are affecting thyroid hormone economy in Great Lakes fish, but there is recent evidence (Leatherland, 1993; Leatherland, 1994) that environmental factors other than EDCs may be responsible for the effects. However, a range of contaminants, especially mercury and petroleum hydrocarbons, in

Piles Creek, New Jersey, have been associated with impaired behaviour and thyroid dysfunction in wild mummichogs (Zhou et al., 2000).

4.5.3 Conclusion

This brief review shows that the endocrine disruption that is undoubtedly occurring in wild fish populations in North America, Asia, Australia and Europe is caused through a variety of mechanisms including hormone receptor interactions, interference with the biosynthesis of sex steroids, and perturbations of the hormonal control by the pituitary on reproductive and adrenal processes. However, in most cases the precise modes of action are still poorly understood and data are largely confined to the gonochoristic species. The compounds responsible for the observed effects may be due to both synthetic and natural compounds. Currently, there is limited understanding of how the existing endocrine disruption affects population fitness.

4.6 Invertebrates

4.6.1 Unique aspects

Most major contemporary groups of invertebrates are the product of an evolutionary lineage distinct from that of the vertebrates. Accordingly, the endocrine systems of most vertebrate groups share little commonality with vertebrates and invertebrates exhibit unique susceptibilities to EDCs. Invertebrates also exhibit a variety of anatomical and physiological traits that are under endocrine control and are not present in vertebrates. For example, many invertebrates have complicated life histories and display various forms of hermaphroditism. Some species also exhibit poorly defined sexual dimorphism. Reproductive cycles can be highly complex involving environmental stimuli, such as light intensity, temperature, desiccation and diet. Also, there are unique aspects of sex determination to be considered in certain species. The sex of an individual is not always determined before or at fertilization but may be influenced by environmental conditions experienced during embryonic or larval development (Stahl et al., 1999). For example, in the echiuran worm *Bonellia viridis,* females release a substance that masculinizes developing larvae. The absence of this substance results in the production of female larvae. Separating the effects of various environmental stimuli from the potential effects of EDCs will pose a significant challenge in many invertebrate species. More conventional regulation of sex differentiation and gonadal development by hormones also occurs in some invertebrates, though the hormone involved differs from those of vertebrates. Environmental compounds with endocrine disrupting properties may influence these hormonally regulated processes, as in vertebrate wildlife.

Consistent with the function of the vertebrate endocrine system, the endocrine system of invertebrates functions to transduce signals, either environmental or endogenous, to appropriate target sites in order to elicit the required response (LeBlanc et al., 1999). These signaling pathways are often initiated with neuropeptides that may directly stimulate the targeted response or may be part of a cascade of hormones, which include non-peptide hormones such as steroid hormones and juvenoid hormones. As in vertebrates, these non-peptide hormones may be more susceptible to endocrine toxicants since exogenous chemicals are more likely to interact with the receptors to these hormones.

Two major classes of hormones in the arthropods (i.e., crusteaceans, insects, and some minor phyla) are the ecdysteroids and juvenoids. These hormones share significant structural and functional homology with the sex-steroids and retinoids of vertebrates. Ecdysteroids are primarily recognized for their regulation of molting, embryo development, diapause, cuticle formation, and various aspects of reproduction including vitellogenin production, ovulation, and spermatogenesis (Wigglesworth, 1984; Hagedorn, 1985; Koolman, 1989). Terpenoids (specifically the juvenoids) have been recognized primarily in insects for their role in promoting juvenile to adult metamorphosis (LeBlanc et al., 1999). Terpenoids are now recognized to function in concert with, and perhaps independently of, ecdysteroids to regulate a variety of functions including reproduction, caste determination, behaviour, diapause, and metabolism (Nijhout, 1998). The action of both ecdysteroids and retinoids is mediated by specific receptors that are likely susceptible to binding by EDCs.

Unique lifestyle and habitat preferences of many invertebrates are influential factors in determining the extent of their exposure to EDCs. Filter feeding organisms are exposed to EDCs through the water phase (and the particulate components in water), while deposit feeders encounter EDCs adsorbed to sediment particles (Depledge and Billinghurst, 1999). For example, deposit feeding organisms like the polychaete worm *Dinophilus gyrociliatus* may be affected by nonylphenol, which accumulates on particulate matter. Controlled experiments demonstrated that exposure of *Dinophilus gyrociliatus* to environmentally realistic concentrations of nonylphenol was associated with increased egg production but reduced egg viability (Price and Depledge, 1998). Herbivorous species potentially ingest a wide range of natural phytoestrogens and mycoestrogens. Interestingly, some phytosteroids are highly soluble in water and are structurally similar (e.g., brassinosteroids) to ecdysteroids (the arthropod molting hormone) (Luu and Werner, 1995). Indeed, Subramanian and Varadaraj (1993) found that molting is arrested in some aquatic insects exposed to paper and pulp mill effluents that contain high concentrations of phytosteroids. As well, carnivorous invertebrates may consume significant amounts of hormone mimics from contaminated prey.

[insert Figure 4.6]

4.6.2 Effects based responses and case studies

4.6.2.1 Effects of tributyl tin (TBT) on gastropods and bivalves

The most complete example of endocrine disruption by an environmental contaminant is documented in molluscs exposed to TBT. TBT is a compound found in anti-fouling paints that are applied to the hulls of ships. In the 1980s, the condition of imposex (the imposition of male sex organs including a penis and vas deferens onto females) was observed with increasing frequency in marine gastropods exposed to TBT (Bryan et al., 1986; Smith and McVeagh, 1991; see Figure 4.7). Supporting work demonstrated that injections of TBT into female common dogwhelks (*Nucella lapillus*) induce penis formation (Lee, 1991; Spooner et al., 1991). The frequency of imposex and degree of penis development are related to the degree of TBT exposure (Bryan et al., 1986). The consequences of imposex in the dogwhelk *Nucella lapillus* include distortions of the sex ratio, reductions in the recruitment of juveniles, and reduced population numbers (Matthiessen et al., 1999). Approximately 150 different species of prosobranch gastropods have been affected by organotins (TBT and triphenyltin) worldwide (Matthiessen et al., 1999). TBT also induces a second masculinization phenomenon in the periwinkle *Littorina littorea*: intersex (Oehlmann, 1998). In this case, the female pallial organs are modified toward a male morphological structure that can lead to the formation of a prostate gland. Both imposex and intersex can result in the sterilization of the females.

Testosterone and the peptide APGWamide have been shown to stimulate male sex organ development in female gastropods (Bettin et al., 1996; Oberdorster and McClellan-Green, 2001). These hormones may function to stimulate male sex organ development. Currently, TBT is not known to influence the activity of APGWamide; however, TBT does elevate testosterone levels in female gastropods (Spooner et al., 1991; Bettin et al., 1996; Matthiessen and Gibbs, 1998). The inhibition of testosterone aromatization or conjugation of testosterone to polar, highly excretable derivatives of testosterone have been proposed as possible mechanisms by which TBT elevates testosterone levels (Spooner et al., 1991; Stroben et al., 1991). Recent studies using the mug snail (*Ilyanassa obsolete*) have revealed that testosterone is conjugated primarily to fatty acids that are retained by the snails (Gooding and LeBlanc, 2001). This is a rather unique mode of testosterone inactivation and the TBT-induced inhibition of the acyl CoA:testosterone acyltransferase responsible for this esterification may prove to be the reason why gastropods are uniquely susceptible to the endocrine disrupting activity of TBT.

[insert Figure 4.7]

In addition to gastropods, TBT has been found to have various effects on the reproduction of several species of bivalve molluscs such as the European flat oyster (*Ostrea edulis*), blue mussels (*Mytilus edulis*), and an intertidal clam (*Scrobicularia plana*) (Matthiessen et al., 1999). The Pacific oyster (*Crassostrea gigas*) develops unusual shell morphology with thickened valves and internal chambers in response to TBT exposure and in Arcachon, France and in the United Kingdom, shell deformities were found to be less severe farther from marinas (Alzieu, 1991). At high concentrations, female oysters exhibit altered reproductive systems and spermatogenesis may occur. While reproductive effects of TBT in bivalves have been linked to alterations in endocrine function, it is unclear whether shell thickening in Pacific oysters is a consequence of endocrine disruption (Matthiessen et al., 1999).

4.6.2.2. Endocrine disruption in crustaceans

4.6.2.2.1 Disruption of ecdysteroid-regulated processes

Ecdysteroids elicit physiological responses through interaction with an ecdysone receptor. Environmental chemicals have the potential to function as ecdysone agonists by binding to and activating the ecdysone receptor. Many plant compounds are known to function as ecdysone receptor agonists (Dinan et al., 2001) and some insecticides function by acting as ecdysteroids (Sundaram et al., 1998). Ecdysteroid agonists have been shown to acclerate molting, cause incomplete ecdysis, and cause death during molting (Clare et al., 1992; Baldwin et al., 2001).

Environmental chemicals also can bind to the ecdysone receptor in an antagonistic fashion. Chemicals shown to function as ecdysone receptor antagonists include bisphenol A, lindane, and diethylphthalate (Dinan et al., 2001). The fungicide fenarimol has been shown to function as an antiecdysteroid in crustaceans by lowering levels of endogenous ecdysone (Mu and LeBlanc, 2001). Consequences of antiecdysteroids exposure to crustaceans include delayed molting and developmental abnormalities (Mu and LeBlanc, 2001). The steroidal androgens testosterone and androstenedione have been shown to elicit antiecdysteroidal activity and this property is likely responsible for the developmental toxicity of these chemicals to crustaceans (LeBlanc et al., 2000; Dinan et al., 2001).

4.6.2.2.2 Disruption of juvenoid-regulated processes

The structure and function of juvenile hormones have been thoroughly characterized in insects. These hormones appear to function through interaction with retinoid X-like receptors (i.e., ultraspherical) that heterodimerize with the ecdysone and perhaps other receptors (Yao et al., 1993; Jones and Sharp, 1997). In crustaceans, methyl farnesoate has functional homology to the juvenile hormones of insects

(LeBlanc et al. 1999). Methoprene is a juvenile hormone analog that elicits a variety of effects in crustaceans that are suggestive of endocrine disruption. Methoprene has been reported to reduce fecundity (Templeton and Laufer, 1983; McKenney and Celestial, 1996; Chu et al., 1997; Olmstead and LeBlanc, 2001a), interfere with juvenile development (Templeton and Laufer, 1983; McKenney and Matthews, 1990; Celestial and McKenney, 1994), reduce growth rate and molt frequency (Olmstead and LeBlanc, 2001a), and delay reproductive maturation (Olmstead and LeBlanc, 2001a). Methoprene also has been shown to stimulate excess male production in *Daphnia magna* (Olmstead and LeBlanc, 2001b). Many of these effects occurred at methoprene levels significantly lower than environmental concentrations measured at application (Knuth, 1989; Ross et al., 1994). Environmental chemicals such as atrazine and 4-nonylphenol also have been shown to stimulate male production in daphnids (Baer and Owens, 1999; Dodson et al., 1999a); while, dieldrin exposure reduced the number of males produced by daphnids (Dodson et al., 1999b). These observations raise the possibility that environmental chemicals may interfere with juvenoid-regulated processes in crustaceans with adverse consequences.

4.6.2.3 Molting disturbances and deformities in insects

There is a large body of literature on the effects of certain chemicals on the insect endocrine system due to the development of insecticides that are designed to alter endocrine functioning. Endocrine disrupting effects of insecticides on nontarget insects have also been reported. For example, treatment of orchards with fenoxycarb (a juvenile hormone analog) has been shown to have deleterious effects on the development of honeybee broods (LeBlanc et al., 1999).

The ecdysteroid receptor agonist, tebufenozide, causes hyperecdysonism in certain insects (Wing, 1988). These effects include the delayed postembryonic development of the diamondback moth (*Plutella xylostella* L.) and the grey fleshfly (*Neobellieria bullata* Parker). Also, tebufenozide induces molting disturbances in Colorado potato beetle (*Leptinotarsa decemlineata* Say) and cabbage butterfly (*Pieris brassicae* Hubner) and causes an early first molt in *N. bullata* and nymphal-adult intermediates in the milkweed bug (*Oncopeltus fasciatus* Dallas). In the latter species, exposure resulted in sterility (Darvas et al., 1992). Darvas also found wing and mandible malformations of insects following exposure to tebufenozide.

Phytoectosetroids have been reported to have hormonal activity in aquatic insects (Adler and Grebenok, 1995). Effluents from pulp and paper mills, which contain phytoectosetroids, have been found to influence molting in dragon fly larvae (Subramanian and Varadaraj, 1993). Exposure of the same

species to tannery effluent resulted in a shortened time to first molt. The authors suggested that components of the effluents might have interfered with ecdysteroid metabolism.

In chironomid larvae, increased incidences of deformities of the mouthparts and other head capsule features may be induced by exposure to sediments contaminated with DDE, heavy metals, and pulp and paper mill effluents (Matthiessen et al., 1999). In controlled laboratory studies, chironomid larvae deformities were induced by exposure to heavy metal concentrations similar to those found in the field (Dickman and Rygiel, 1996). While the underlying mechanism responsible for these effects are unknown, the developmental alterations that occur have led to the hypothesis that an endocrine based mechanism is involved.

4.6.3 Conclusion

The diversity of the invertebrate phyla creates numerous challenges in determining the potential risks of EDCs to the health of these animals. Compounding these challenges is the poor understanding of the endocrinology of most invertebrates, and even though the endocrinology of arthropods is best understood among invertebrates, gaps in our knowledge still exist. It is clear that the effects of EDCs in vertebrates will not necessarily be similar to those observed in invertebrates. Conversely, invertebrates are susceptible to endocrine-disrupting properties of compounds that are not problematic in vertebrates. More field-based studies are required to determine the extent to which invertebrate species, both aquatic and terrestrial, have been impacted by exposure to EDCs in their environment.

4.7 Uncertainties And Research Needs

The case studies reported in this chapter have provided strong evidence that there are effects observed in wildlife that can be attributed to substances that function as EDCs. However, there are a large number of situations where the evidence of a causal link with endocrine disruption is weak or non-existent. It is apparent that the variety of responses in wildlife that can be attributed to EDCs are most obvious in species inhabiting areas, which have received extensive chemical contamination. There are unanswered questions as to whether areas contaminated with lower levels (i.e., background levels) of compounds also pose significant risks to wildlife or, are the ranges of effects restricted to the wildlife studied this far.

The case studies illustrate some of the challenges encountered when establishing cause and effect relationships between chemical exposures and physiological dysfunction in diverse species. All

ecotoxicological investigations are complicated by a variety of factors that may impact growth, reproduction, and survival. For example, food availability, disease state, competition, and loss of habitat are significant stressors to wildlife and impinge on many of the endpoints measured in investigations that assess the risks posed to wildlife by EDCs. Other confounding factors relate to the lack of knowledge of the endocrine, reproductive and developmental biology in many wildlife species. While it is recognized that there are critical stages of embryonic developmental that may be especially impacted by EDCs, few studies have explored this issue. Given the diversity of wildlife, it may be inappropriate to extrapolate the responses to EDCs and other stressors, as research has focused predominantly on only a few species. Currently, routine ecotoxicological assessments tend to focus only on a few species of wildlife. This approach also limits the ability to assess the ecological relevance of anthropogenic influences on the environment. As an example, one group of avian species that has been under represented is passerine birds. This has occurred despite evidence of widespread declines in populations of several species of songbirds. Similarly, there have been few studies that have considered invertebrates despite the knowledge that invertebrates are key to the structure and function of ecological systems.

Most ecological risk assessments of wildlife tend to focus on populations and communities. However, when considering the potential effects of EDCs to wildlife, the focus tends to be on the individual. This may be problematic as there is a limited understanding of how physiological changes affect the individual or how individual responses affect population and community outcomes. The ecological significance of disturbances in growth, reproductive output, viability of offspring, altered sex ratio, and of potential transgenerational effects is difficult to quantify. One area of investigation that merits consideration is the possibility that artificial selection has occurred as a result of multigenerational exposure to EDCs.

Compared to common laboratory models, many wildlife species are difficult to sample in a systematic fashion due to their lifestyle. To determine the extent of EDC-induced effects (e.g., population decline, malformations) on some species of wildlife, long-term monitoring is needed that would provide baseline data on population status. This type of research would be instrumental when attempting to delineate the relative risks of EDCs from other stressors and for perspective analyses associated with the release of new chemicals in the environment.

Given the breadth of responses seen in wildlife to date, it is imperative that research continues to address the extent of risk posed to wildlife by EDCs. Even though, as far as ecological risk assessments are concerned, discussion of the "endocrine disruptor hypothesis" is somewhat of an artificial

classification. The critical issue is to assess the status of wildlife populations in terms of possible effects on growth, reproduction, and survival. This will necessitate collaboration and cooperation on an international scale to identify populations that are at risk, to dedicate the resources to address critical knowledge gaps, and to ensure that the most current and up to date methodologies in endocrinology, physiology, ecology, and epidemiology are used when evaluating effects on wildlife. Finally, we need to communicate the results of studies widely and effectively such that the true risks posed by EDCs are understood and that policy decisions and regulatory practices are based on the best scientific knowledge.

Figure Legends

Figure 4.1. Eggshell thickness index [shellweight/(shell length x shell breath x shell thickness)] of guillemot (*Uria aalge*) eggs from the Baltic during 1969 –1989 (blue line). The red line indicates the eggshell thickness index of pre 1946 guillemot eggs from the same nestling colony (modified from Bignert et al. 1994).

Figure 4.2. Mean phallus size in alligators (*Alligator mississippiensis*) from two regions from a control lake (Lake Woodruff, n=40) and two regions of a contaminated lake (Lake Apopka). Lake Apopka samples are separated into two localities, the Gourd Neck Spring area (Apopka-GNS, n=34) where contaminants from the Tower Chemical Spill entered the lake and the northwestern part of the lake (Apopka-NW, n=20), which is farther from the spill. Penis size is represented as an index ((penis tip length X penis base width)/ snout vent length). Values from each of the different lake regions are significantly different. Redrawn from Guillette et al. (1996).

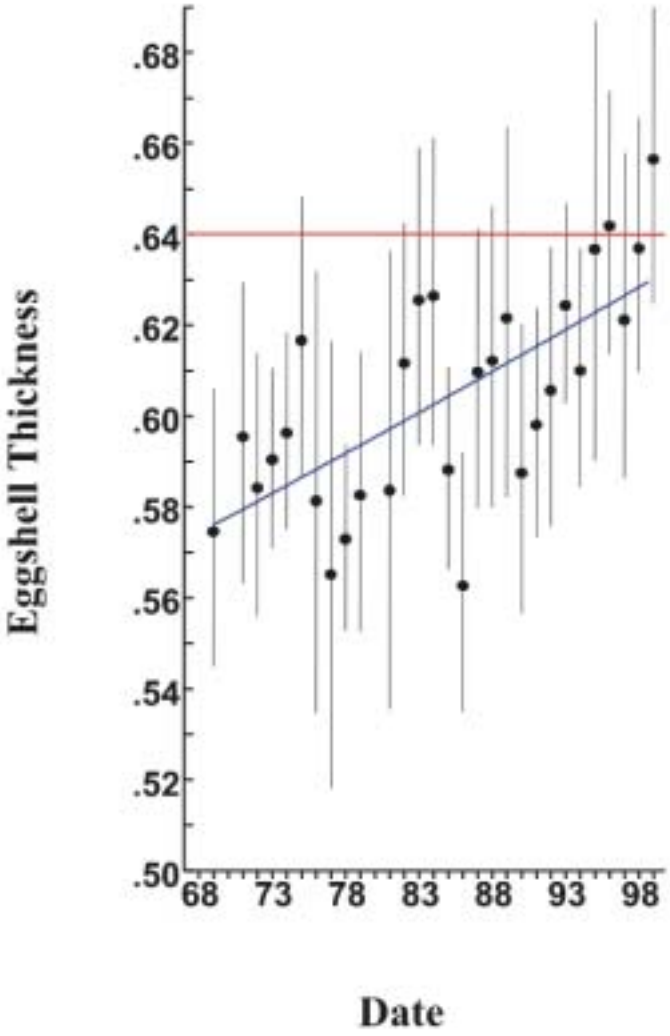
Figure 4.3. Mean concentrations of plasma vitellogenin (VTG) in male flounder (*Platichthys flesus*) from various United Kingdom estuaries sampled in 1997. Bars indicated by an asterisk are significantly different from the clean reference estuary, Alde (extreme left). Reproduced by permission of the United Kingdom Centre for Environment, Fisheries, and Aquatic Science (CEFAS).

Figure 4.4. Testis section from a male flounder (*Platichthys flesus)* sampled in the United Kingdom Mersey estuary in 1996, showing several large secondary oocytes along side abnormal sperm cell tissue (ovotestis). Reproduced by permission of the United Kingdom Centre for Environment, Fisheries, and Aquatic Science (CEFAS).

Figure 4.5. Ovaries from white sucker (*Catostomus commersoni*) collected during their spawning migration. The fish on the left was from a population living in a remote undeveloped region of Lake Superior (Mountain Bay). The fish on the right was from a population living in Jackfish Bay, Lake Superior, which receives bleached kraft pulp mill effluent (BKME) from a mill at Terrace Bay, Ontario, Canada. Ovarian size was significantly reduced in fish originating from the BKME exposed site. Pictures provided by Drs. M. McMaster, K. Munkittrick, and G. Van Der Kraak.

Figure 4.6. Lake trout (*Salvelinus namaycush*) sac fry exposed as a fertilized egg to control vehicle (top) or to TCDD in vehicle (bottom). The bottom fry shows signs of blue-sac disease including yolk sac and pericardial edema, subcutaneous hemorrhages, and craniofactial malformations. Reprinted from Cook et al. (1997).

Figure 4.7. Imposex in the common whelk (*Buccinum undatum* L). Panel A shows a female whelk without imposex; the head and tentacles (to the right) the body is smooth; extended foot to the left lower side of the picture. Panel B shows a female with imposex: the tentacles (to the right) a skin protrusion has developed; this is a sign of imposex, and is located at the right side of the body, below the right tentacle. Pictures provided by Dr. C.C. ten Hallers-Tjabbes, H. Kralt, Dr. J.P. Boon, Royal Netherlands Institute for Sea Research.



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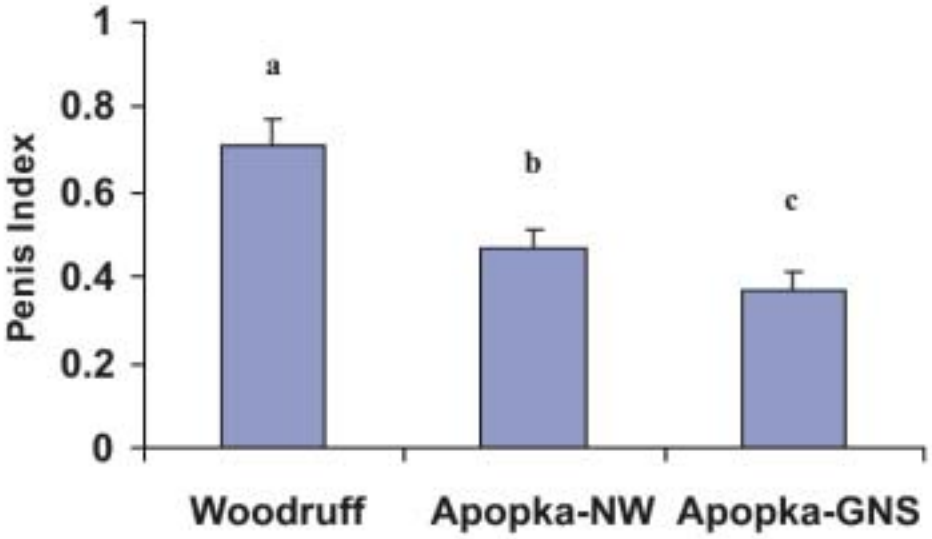

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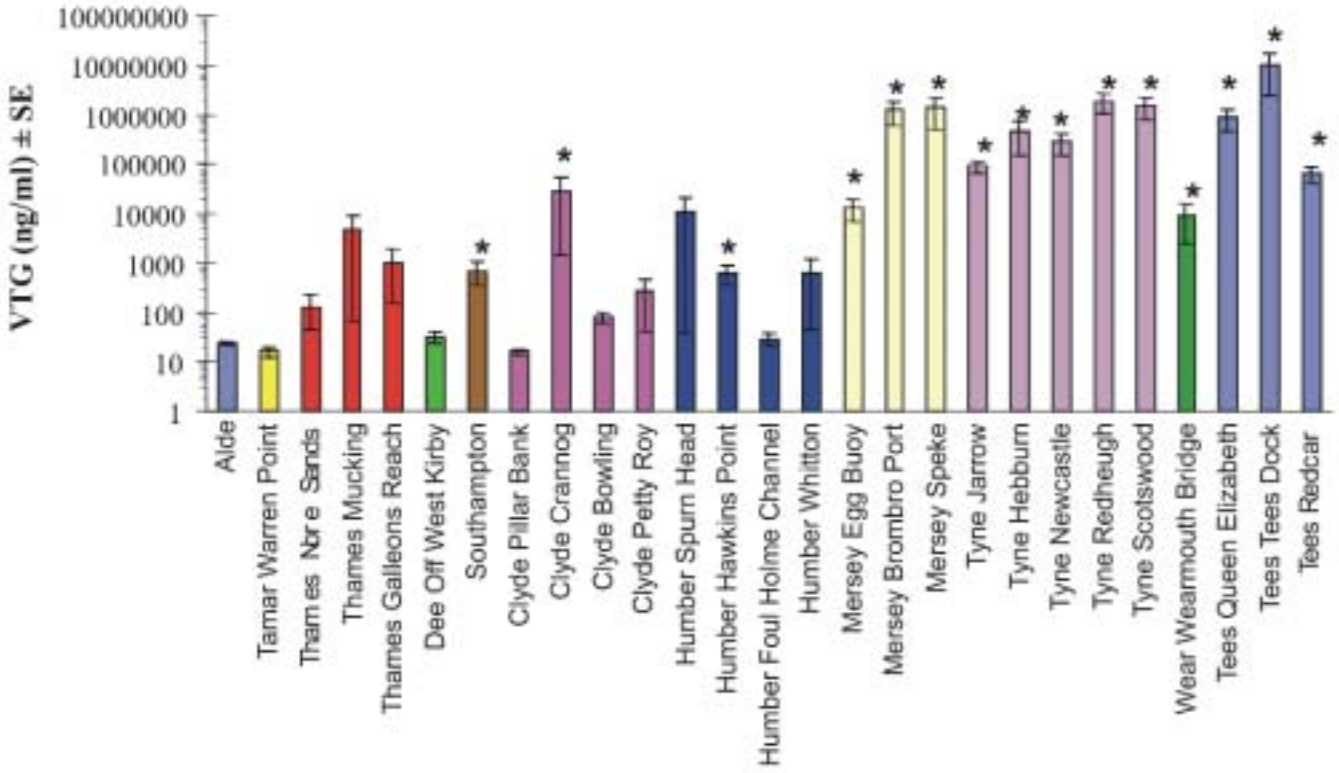

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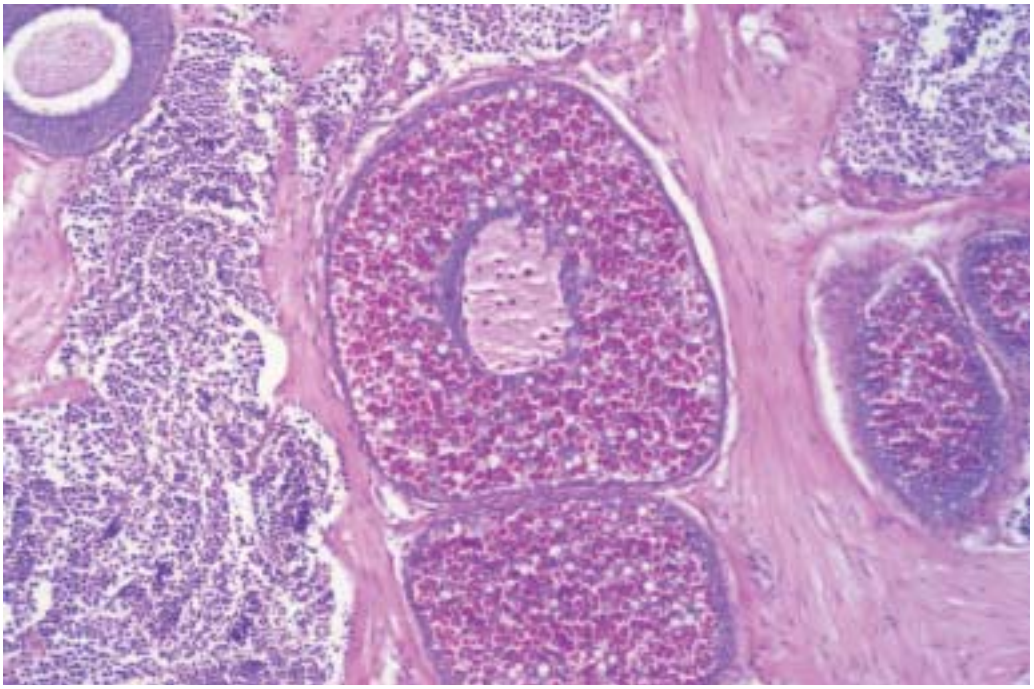

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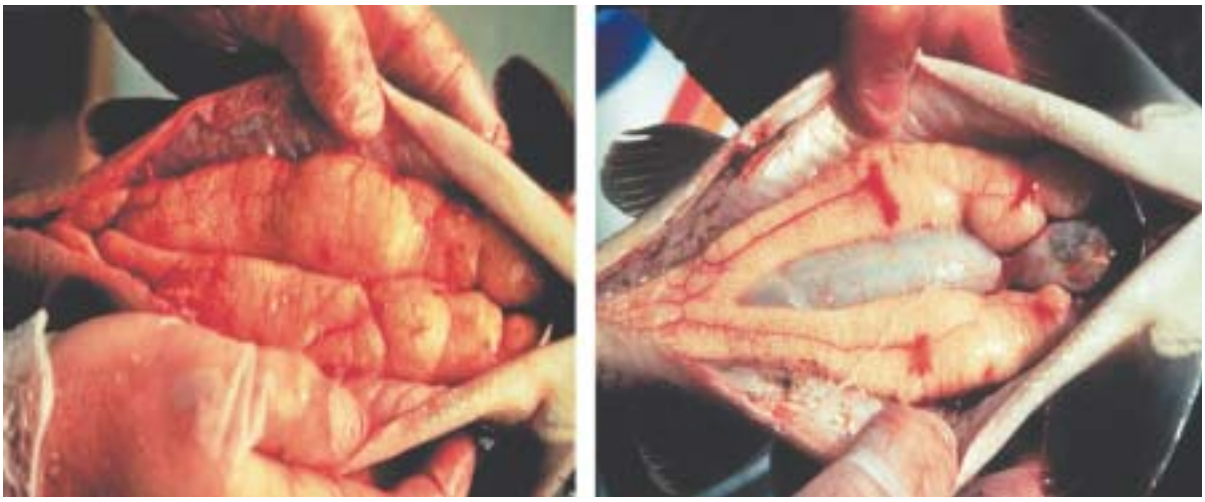

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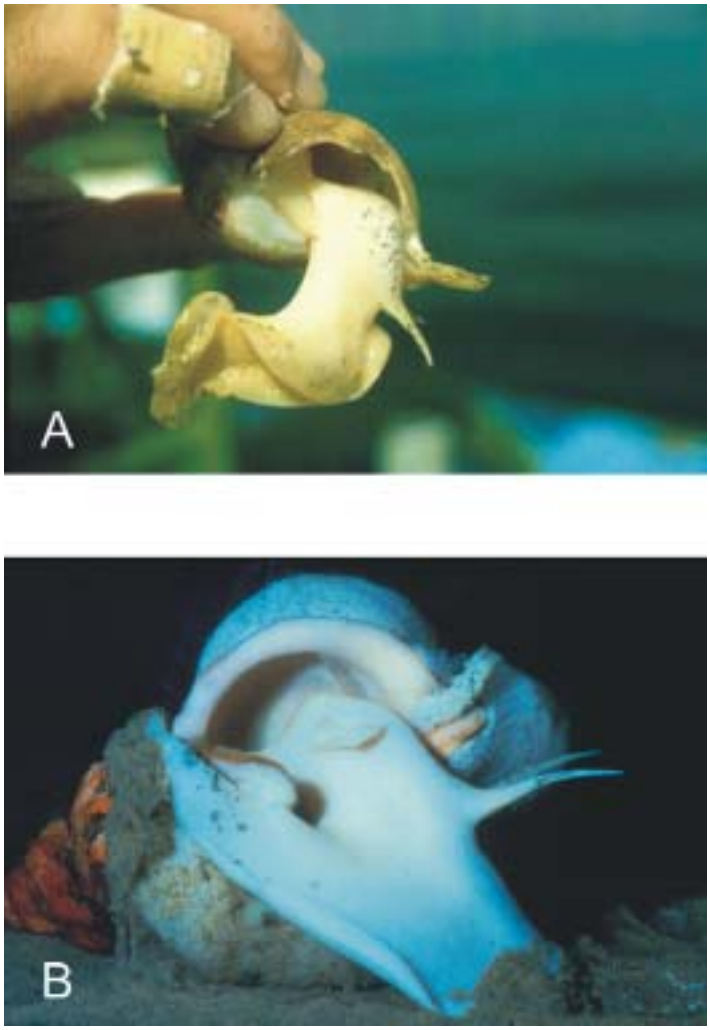

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